GR

ORIGINAL SUBMISSION

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September 3, 2008

Dr. Robert L. Martin
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for High-Selenium Yeast

Dear Dr. Martin:

On behalf of Cypress Systems, Inc. of Fresno, CA, we are submitting for FDA review a GRAS notification for High-Selenium Yeast. The attached documentation augments the information that had previously been identified as GRN 241. Questions raised and clarification requested by the agency during April 2008 telephone discussions as managed by Dr. Carrie Hendrickson McMahon have been thoroughly addressed in this resubmission.

In previous discussion with FDA personnel in December, 2007, the question was raised as to the possible applicability of Section 912 of the Food, Drug, and Cosmetic Act. The response to this inquiry is reproduced below for your convenience in applying it to the subject submission.

Section 912 Considerations. Section 912 prohibits the introduction or delivery for introduction of foods into interstate commerce if such foods contain an added FDA-approved drug or an (unapproved) added drug for which substantial clinical investigations have been instituted and for which the existence of such investigations has been publicly disclosed. The amendment goes on to state that such prohibition does not apply if the drug in question was marketed in food before the drug received FDA approval and if such marketing occurred prior to the institution of any substantial clinical investigations involving that drug.

Application to High-Selenium Yeast. The high-selenium yeast that is the focus of the GRAS notification is not subject to the Section 912 prohibition since the high-selenium yeast was introduced into foods (1) prior to the initiation of clinical trials, and (2) well in advance of the public release of information regarding clinical investigations with this material.

Dr. Lon Baugh developed the high-selenium yeast in 1979-1980 while working with Dixie Yeast (which was aquired by Fleischmann's Yeast in 1985), and shortly thereafter the high-selenium yeast was sold in the US as a nutritional supplement through Nutrition 21. In the mid-1980s, Professor Larry Clark at the Arizona Cancer Center located at the

University of Arizona began the multi-center clinical investigation into the possible cancer prevention benefits of high-selenium yeast nutritional supplement, in concert with several colleagues who constituted the Nutritional Prevention of Cancer Study Group. The randomized, double-blind, placebo-controlled clinical investigations implemented under Clark's direction extended over several years, during which time there were no public releases of the results, in part to preserve the integrity of the double-blinded nature of the investigation. In late December of 1996, the results of the Clark study were

published in JAMA (Clark, et al, (1996), JAMA, 276(24), 1957-1963). By this time, high-selenium yeast had been in the US marketplace as a dietary supplement for over 15 years.

Conclusion. Since the high-selenium yeast is not an-FDA approved drug and since it was introduced into the US marketplace as a dietary supplement prior to the initiation of clinical investigations as to its cancer prevention applications, and furthermore that there were no public disclosures of the clinical trials until late 1996, Section 912 of the Food, Drug, and Cosmetic Act **does not prohibit** the addition of GRAS designated high-selenium yeast into foods that would enter interstate commerce in the US.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

Robert S. McQuate, Ph.D.
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Enclosure: GRAS Notification – High Selenium Yeast (in triplicate)

GRAS ASSOCIATES, LLC

GRAS ASSESSMENT

OF

HIGH-SELENIUM YEAST

Food Usage Conditions for General Recognition of Safety

For

CYPRESS SYSTEMS, INC.

Evaluation By

Robert S. McQuate, Ph.D.
Richard C. Kraska, Ph.D., DABT

September 2, 2008

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

High-selenium yeast (*Saccharomyces cerivisiae)*, meeting the specifications described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by experts qualified by scientific training and experience; it is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the ingredient's intended use in foods.

Signed:

_____

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074 Date:_____

B. Name and Address of Notifier

Cypress Systems, Inc.[2]
3381 North Bond Avenue, Suite 101
Fresno, CA 93726

As the notifier, Cypress Systems, Inc. accepts responsibility for the GRAS determination that has been made for high-selenium yeast as described in the subject notification; consequently, high-selenium yeast meeting the conditions described herein is exempt from pre-market approval requirements for food ingredients.

C. Common Name and Identity of the Notified Substance

High-selenium yeast; also see Sections II.B and II.C.

[1] See 62 FR 18938 (17 April 1997).
[2] Cypress Systems, Inc. ("CSI") produces and sells SelenoExcell®, a high-selenium yeast product.

D. Conditions of Intended Use in Food

High-selenium yeast is intended to be added to the following food categories at a level yielding 5 µg selenium per serving: baked products; non-alcoholic beverages; breakfast cereals; grain products & pastas; milk products; processed fruits/fruit juices; processed vegetables/vegetable juices; commercial soups & soup mixes; and medical foods. Foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, are excluded from the list of intended food uses of the subject high-selenium yeast.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, high-selenium yeast has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. DESCRIPTION OF INGREDIENT

A. Background on Selenium and SelenoExcell®

The role of selenium in biological systems is both intriguing and complex. Regarding health and physiological implications, selenium was first recognized as having toxic properties in animals in the mid-1900s and was later determined to be a trace mineral that is an essential human nutrient, as discussed by Combs (Combs, 1997). An impressive collection of studies has noted the cancer prevention characteristics of certain forms of selenium (Combs, et al., 2001 & Uden, 2004). In particular, epidemiological studies involving subjects from 27 countries reveal an inverse relationship between selenium intake and the incidence of certain types of cancer (Whanger, 2004). This conclusion was strongly supported by a 1990 Finnish study that investigated nearly 40,000 subjects over more than a decade and concluded that selenium intakes that are too low may place subjects at increased risk of some cancers (Knekt, et al., 1990).

Selenium deficiency has also been observed in humans. Keshan disease, an endemic cardiomyopathy, has been reported in selenium-deficient regions of China. Kashin-Beck disease, a musculoskeletal disorder, muscular pain and muscular and cardiac dysfunction were noted in some patients, while others with insufficient selenium status have experienced compromised immune function and viral infection (European Commission, 2000, & Expert Group, 2003).

Selenium occurs naturally in a variety of common foods, including cereals such as corn, wheat and soybeans and other foods such as broccoli, onions, garlic, eggs, seafood, and Brazil nuts (Expert Group, 2003). Various foods with quantitative information on their selenium contents are compiled elsewhere (National Institutes of Health, 2006, Rayman, 2004, Rayman, 2008, & Rayman, et al., 2008). Cereal grains and enriched yeast contain selenomethionine as the predominant form of selenium whereas Se-methylselenocysteine is the major selenocompound found in onions, garlic, and broccoli (Whanger, 2004 & Whanger, et al., 2000).

Selenium's natural occurrence on the earth's surface is highly variable, with higher levels of selenium found in portions of the Great Plains in the US and selected regions of China. Other areas, such as the southern island of New Zealand and parts of China and Europe, are characterized as having particularly low levels of selenium. Both geology and geography have historically played appreciable roles in achieving dietary sufficiency of selenium since the selenium content varies depending on the selenium content of the soils where plants are grown. Locations where the soils are deficient in selenium tend to yield foods that have low levels of selenium which contribute toward a selenium deficiency status in humans.[3]

Inorganic forms of selenium, such as sodium selenite, sodium hydrogen selenite, and sodium selenate (Infante, et al., 2005), that are found in soils are taken into plants where they are converted into organoselenium species such as selenocysteine, selenomethionine, selenium-methylselenocysteine, and γ-glutamyl-Se-methylselenocysteine (Ip, et al., 2000, Bird, et al., 1997, Whanger, 2004, Rayman, 2008, &

[3] With advanced and efficient food distribution systems that are in place in the US (and in other advanced countries worldwide), there is diminishing "regionalization" of foods where compositional contents reflect the local or regional growing conditions. The increasing "nationalization" of the US food supply is tending to level out the selenium deficiencies and excesses that otherwise would be manifested by geological and geographical considerations. Even so, there remains variability in selenium content in foods grown in different geographic regions, and this especially remains the case in less developed nations where diets reflect more localized growing circumstances. Dietary deficits and excesses are more likely to occur in these areas (Rayman, 2008).

Rayman, et al., 2008). Selenomethionine and selenocysteine are considered to be the most common forms of selenium contained in foods (Expert Group, 2003 & European Food Safety Authority, 2008). Upon ingestion by man and animals, such organoselenium forms become incorporated into a number of important selenoproteins and enzymes which, among other functions, yield an antioxidant capability to help reduce cellular damage due to free radicals (National Institutes of Health, 2006).

According to Combs, it was not until 1957 that indications of a positive health role for selenium were established, and additional studies conducted during the intervening years have demonstrated convincingly the essentiality of selenium.[4] By the 1970s, it was conclusively determined that selenium played an essential role in the enzyme glutathione peroxidase which participates in the antioxidant protection of cells (Combs, 1997). By 2003, over 30 selenoproteins were identified (Expert Group, 2003). Table 1 lists several health consequences of consuming adequate to somewhat elevated amounts of dietary and supplemental selenium.

Table 1. Claimed Favorable Health Effects Ascribed to Selenium

Reduced risks of certain types of cancers (a), (b), (c)
Provide metabolic defense against oxidative stress (a), (b), (c)
Support male prostate function (a)
Reduce menopausal symptoms in females (a)
Provide hormonal regulation of energy metabolism (b)
Enhance immune response (a), (c)
Enhance fertility & reproduction (c)
Reduce inflammation (c)
Alleged protection against bird flu (d)
Treat HIV infection (c), (e), (f)
Maintain intracellular redox state (f)
Improve skin disorders (f)
Alleged benefit to elderly women in inhibiting lipid peroxidation (g)
Protect against exposure to heavy metals (a), (h)

(a) Schauss, 2006.
(b) Combs, 2000.
(c) Rayman, 2004.
(d) http://www.nutraingredients.com /news/ng.asp?n=66089&m=1NIE227&c=fjbaapnlrdryleu.
(e) http://www.healthy.net /scr/interview.asp?PageType=Interview&ID=198.
(f) Expert Group, 2003; (g) http://www. nutraingredients.com/news/ng.asp?n=64658&m=1NIED19&c=fjbaapnlrdryleu.
(h) http://www. nutraingredients.com/news/ng.asp?n=65560&m=1NIE202&c=fjbaapnlrdryleu

Since selenium is a normal constituent of both plant- and animal-derived foods, it has been consumed for generations world-wide (Combs, 1997). Rayman compiled estimated selenium intake levels for individuals in various countries along with the minimum recommended selenium intake levels for adults in certain

[4] The micronutrient status of selenium has been well-established, and from a historical standpoint, as noted in Table 1, various purported health benefits have been linked to dietary selenium. As noted in Sections III. and IV., health benefits associated with dietary selenium continue to be a topic of much interest to the scientific community. Numerous studies have been conducted and continue to be investigated in the search for positive health outcomes, In noting this, it is understood that food ingredient safety determinations, such as GRAS determinations, are based solely on safety or risk considerations and **not on risk/benefit analyses**. Those studies addressing benefits, such as the cancer prevention trials addressed by Clark, et al., 1996 and various subsequent or continuing investigations, are included because of the safety considerations that can be derived from them.

countries. These data are consolidated in Table 2 (Rayman, 2004; Rayman, 2008; & Rayman, et al., 2008). Recommended Intakes have been established to be no less than 30 µg/day per person and is more commonly in the 50–70 µg/day range. Actual selenium intake by individuals across several countries reveals adequacies in Japan, Venezuela, and Canada while other nations, especially those in Europe, consume suboptimal levels falling in the range of 10–67 µg/day (Rayman, 2004 & Rayman, 2008). China is interesting in that it has regions where there is a severe selenium deficiency while other regions have high selenium concentrations in the soil. Consequently, selenium intake data in China have been estimated to fall in the range of 7–4990 µg/day.

Table 2. Actual Selenium Intakes and Recommended Selenium Intakes[a]

Geographic Location	Selenium Intake Per Person (µgrams/day)	Recommended Intakes (µgrams/day) Males	Females
Australia	57 – 87	85	70
Austria	48	30 – 70	30 – 70
Belgium	28 -- 61	70	70
Canada	98 – 224	55	55
Czech Republic	10 – 25		
China	7 – 4990		
Croatia	27		
Denmark	38 -- 47		
EC Scientific Committee		55	55
France	29 – 43	60	50
Germany	35	30 – 70	30 – 70
Italy		55	55
Japan	104 – 199	55 – 60	45
Netherlands	39 – 67		
New Zealand	55 – 80	65	55
Nordic Countries		50	40
Poland	30 – 40		
Serbia	30		
Slovakia	38		
Sweden	31 – 38		
Switzerland	70	30 – 70	30 – 70
United Kingdom	29 – 39	75	60
United States	106	55	55
Venezuela	200 – 350		
World Health Organization		40	30

[a] From Rayman, 2004 and references cited therein; also see Rayman, 2008.

In the US, the Dietary Reference Intake for selenium, which is viewed as an "adequate intake," has been established for male and female adults to be 55 µg/day per person (Food and Nutrition Board, 2000), while the average intakes are nearly double that amount. Schrauzer also reports that selenium intake for the average adult in the US is 80–150 µg/day, exceeding by a factor of about 2 the adequate intake amounts in the US (Schrauzer, 2001). Furthermore, the Tolerable Upper Intake Levels (ULs) in the US (to be discussed in more detail in Section IV) for adult males and females was established to be 400 µg/day (Food and Nutrition Board, 2000). (See Table 3 for more detail on the Dietary Reference Intakes and Tolerable Upper Intake Levels in the US.)

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Along with the documentation that selenium deficiencies exist in parts of the world, it has also been well-established that selenium can be toxic when consumed at higher levels. Therefore, we note that there is a range of acceptable selenium intakes, below which there are adverse health consequences due to selenium deficiencies and above which selenium toxicity is observed.

Table 3. US Reference Daily Intake (RDI) and Tolerable Upper Intake Levels (UL) for Selenium[a]

Life Stage Group	RDI (µg/d)	UL (µg/d)
Infants		
0 – 6 mo	15	45
7 – 12 mo	20	60
Children		
1 – 3 y	20	90
4 – 8 y	30	150
Males		
9 – 13 y	40	280
14 – 18 y	55	400
19 – 30 y	55	400
31 – 50 y	55	400
50 – 70 y	55	400
> 70 y	55	400
Females		
9 - 13 y	40	280
14 – 18 y	55	400
19 – 30 y	55	400
31 – 50 y	55	400
50 – 70 y	55	400
> 70 y	55	400
Pregnancy		
< 18 – 50 y	60	400
Lactation		
< 18 y – 50 y	70	400

[a] Extracted from Food and Nutrition Board, 2000.

Understanding the nutritional and toxicological ramifications of any element depends on the particular chemical forms of that element, along with their relative quantities and possible chemical interactions.

Therefore, assessing the appropriate dietary intake levels of selenium requires consideration of the actual selenium levels and the form(s) of selenium to be ingested. SelenoExcell® is a high-selenium yeast and serves as the primary focus of this evaluation.[5] Even so, the technical information on inorganic selenium and other readily available forms of this trace mineral, especially selenomethionine, contributes appreciably to the subject safety assessment. One of the complexities to be considered in assessing safe dietary levels of selenium involves identifying the chemical forms of selenium that are present (Uden, et al., 1998 & Rayman, et al., 2008). In fact, Bird, et al., unequivocally state that the nutritional bioavailability, toxicity, and cancer chemopreventive activities of selenium have been found to be species-dependent (Bird, et al., 1997), and this was reinforced more recently by Rayman and co-workers (Rayman, 2008 & Rayman, et al., 2008). Furthermore, the metabolic conversions of the different selenium forms are of interest in this evaluation, as are the differing degrees of bioavailability.

B. Chemical Name and Common or Usual Name of the Subject Material

As reported in Section I.C., high-selenium yeast is the common name of the notified substance, and SelenoExcell® High Selenium Yeast is the commercial name of the subject material for which the GRAS evaluation has been undertaken. As discussed more fully in the following section, SelenoExcell® is a mixture of four chemically characterized organoselenium forms which account for just over 85% of the available selenium. Selenomethionine is the predominant form of selenium in the mixture.

C. Chemical Composition

SelenoExcell® and other commercial selenium-enriched yeasts have been investigated by Uden and colleagues at the University of Massachusetts to ascertain their respective chemical compositions. Results of these studies have been reported recently (Uden, et al., 2004 & Uden, et al., 1998). Infante, et al., have reviewed analytical strategies and procedures with a particular focus on selenium forms within complex mixtures such as are found in high-selenium yeasts, high selenium garlic, high selenium onions, and Brazil nuts (Infante, et al., 2005 & Rayman, et al., 2008).

Uden, et al., and Infante, et al., describe treatment of the subject materials with proteolytic enzymes followed by extraction procedures before applying the rigorous detection methods to yield the tabulated results. A combination of procedures---fluoracidic ion pair HPLC with an inductively couple plasma mass spectrometer, along with GC derivatization linked with atomic emission detection---was employed.

Table 4 identifies the selenium forms and amounts found in SelenoExcell® as studied by Uden and colleagues. The study also reports the presumed absence of other known organoselenium forms that have been found in other high-selenium yeast products. Selenomethionine was shown to be the predominant selenium species in SelenoExcell®, as was the case with all other high-selenium yeast products tested. It was also noted that inorganic selenite was present in very low amounts (~0.1%), and this would be expected from the water washing steps noted in the manufacturing process as described in Section II.E. Method of Preparation. Replicate determinations with SelenoExcell® demonstrated a high degree of reproducibility (Uden, et al, 2004).

[5] Once it was established that supplementing the diet with selenium had the potential to impart improved health, different forms of selenium became commercially available. Besides SelenoExcell®, other high-selenium yeasts and other organoselenium forms that are produced in Canada, Europe, and the US, are available, as are inorganic selenium salts. The chemical compositions vary, depending on the manufacturer/supplier (Rayman, 2004).

Nearly 15% of the selenium content in SelenoExcell® has not been characterized. This aspect can conceivably have a bearing on the safety assessment of the subject commercial selenium mixture. Comparable findings were observed with all of the high-selenium yeast products that were tested. Of the six different commercially available high-selenium yeast products tested compositionally by Uden, et al., the total selenium accounted for ranged from a low of 67% to a high of 88%. Rayman has explained that this observation is due to the challenges with inherent analytical limitations including variable extraction techniques (Rayman, 2004).

Table 4. Selenium Forms in Commercially Available SelenoExcell®[a]

Selenium forms	Percentage
Selenomethionine	84
Selenite	0.1
γ-Glutamyl-Se-methyl-Se-cysteine	0.5
Se-adenosyl-Se-homocysteine	0.5
Se-lanthionine	nd/nr
Se-methyl-Se-cysteine	nd/nr
Se-cystathionine	nd/nr
Se-cystine	nd/nr
Se-cysteine	nd/nr
Selenomethionine-selenoxide (or its hydrate)	nd/nr
Methaneseleninic acid	nd/nr
Sum of Identified forms	**85.1**

nd = not detected; nr = not reported

[a] From Uden, et al., 2004 & Rayman, 2004.

D. SelenoExcell® Stability

While there are no indications that the subject material is unstable during normal conditions based on internal quality assurance records, the question of high-selenium yeast stability was recently raised by Uden, et al., who conducted compositional testing of the test materials that had been used by Clark, et al., in the 10+ year cancer clinical testing (Clark, et al, 1996). This highly regarded clinical testing utilized SelenoExcell® as the administered form of selenium that yielded favorable clinical results. According to Uden, et al, the tableted form of SelenoExcell® was stored at room temperature for more than ten years[6], and testing results revealed that a substantial amount of selenomethionine selenoxide hydrate was present. Since this component---presumed to be an oxidation product---was unreported prior to the extended period of time in uncontrolled storage, it is apparent that some conversion of selenomethionine had occurred, at least over an extended period of storage without well-controlled storage conditions (Uden, et al., 2004). However, there is no reason to expect that this has any significant relation to safety.

[6] Considering that the actual clinical trial lasted ten years and the results were published in 1996, actual samples tested could very well have been twenty years old when the speciation testing was conducted.

E. Method of Preparation of SelenoExcell®

Demirci & Pometto published details on the means of producing organically-bound-selenium yeast via continuous aerobic fermentation (Demirci & Pometto, 1999). The process utilizes *Saccharomyces cerevisiae* as the yeast into which inorganic selenium, as Na_2SeO_3, is incorporated. The so-called continuous fermentation utilizes a medium with minimal sulfur and methionine levels to enhance the degree of selenium incorporation into the mother yeast. The production principles described by Demirci & Pometto portray the fundamental continuous fermentation process that is utilized by CSI in producing SelenoExcell®. Rayman also discusses the production processes for various selenium-enriched yeasts and the quality and compositional features resulting from such preparations (Rayman, 2004). CSI describes its particular production process and QA/QC program in summary sheets that are found in Appendix A. Key components are noted below:

- The method utilizes standard baker's yeast strains---*Saccharomyces cerevisiae*---in all of its production.[7]

- The aerobic fermentation is precisely controlled to maintain yeast growth, nutrient feed streams, dissolved oxygen, pH, temperature and the presence of alcohol. Such control was found to yield optimal growth conditions with the proper uptake of selenium.

- Na_2SeO_3 is added to the fermentation vessel at specific times to maximize selenium incorporation into the yeast.

- The resulting primary grown high protein yeast, which is fortified with biologically bound selenium, is separated from its growth medium, washed, and held in refrigerated storage to assure cell viability. The washings are effective in removing free minerals.

- The chilled mineralized yeast cream is inactivated when pasteurized through a high temperature sterilization system to achieve human food grade microbial standards, after which the material is spray dried to yield a uniformly homogeneous dry powder.

- As part of its QA/QC program, CSI collects composite samples during the spray drying phase and during packaging for nutrient and microbial analyses. CSI utilizes Silliker Laboratories for all nutrient and microbiological testing of SelenoExcell®.

- The resulting SelenoExcell® is 99.9% organically bound with residual inorganic selenium levels of 0.1% or less; the controlled process yields minimal batch-to-batch variations.

[7] CSI reports that this particular yeast is considered within the industry to be GRAS. In fact, selected dried yeasts, including *Saccharomyces cerivisiae*, are approved food additives as noted in 21 CFR 172.325. Other food additive regulations including 21 CFR 172.590 and 172.898 authorize additives that are derived from *Saccharomyces cerivisiae*. No FDA documentation was located that explicitly identifies the subject yeast to be **GRAS** except for a recent designation that the genetically modified strain, ECMo01, was concluded to be GRAS. It was determined (see http://www.cfsan.fda.gov/~dms/opa-enzy.html) that the enzyme, invertase, when derived from *Saccharomyces cerivisiae* was considered by FDA to be GRAS based on an FDA opinion letter issued in the early 1960s.

F. Finished Product Specifications and Physical Characteristics

(1) SelenoExcell® Specifications

The specifications shown in Table 5 were developed by CSI. These food grade specifications were established in concert with the National Cancer Institute's Cancer Prevention Division who wanted a standardized composition of high-selenium yeast before selenium-derived materials would be accepted for NCI-sponsored clinical trials. CSI specifications for SelenoExcell® as shown in Table 5 were accepted as part of the 1998 Clinical Trial Agreement executed between CSI and the Cancer Prevention Division.

Table 5. Specifications for SelenoExcell®

Species: *Saccharomyces cerivisiae*	
Items:	
Selenium	1,140 – 1,260 ppm
Protein	49 – 55 %
Phosphorus (measured as P_2O_5)	2.0 – 3.4 %
Moisture	2.5 – 7.5 %
Extraneous material	negative
Inorganic selenium	~0.1%[a]
Microbiology:	
Salmonella	negative
E. Coli	negative
S. aureus	negative
Bacillus cereus	negative
Total coliforms	less than 0.3/gram
Total plate count	less than 10/gram
Yeast/mold	less than 10/gram
Heavy Metals:	
Lead	less than 1 µg/gram
Arsenic	less than 1 µg/gram
Cadmium	less than 1 µg/gram
Mercury	less than 1 µg/gram

[a] The production process for high-selenium yeast involves sufficient water washings to yield negative results when testing with methylene blue indicator. Such an endpoint correlates with ~ 0.1% free inorganic selenium.

Appendix B contains a typical Certificate of Analysis for SelenoExcell®.

(2) Physical Characteristics

Color:		tan
Bulk density:		0.6515 g/mL
Particle size:	through 60 mesh	100%
	through 100 mesh	100%

(3) Yeast Composition and Performance Comparisons

Both the chemical composition and yeast performance parameters were compared to establish that the unmodified yeast and the yeast with incorporated selenium that complies with the specifications detailed in Table 5 were virtually the same except for the significant presence of the selenium in place of sulfur (see below) within the yeast.

According to Dr. Lon Baugh who developed the method of producing SelenoExcell®, the selenium-yeast and the standard baker's yeast can be compared as noted below (Baugh, 2008):

- No significant differences were observed in the yeast growth rates, respiration rates, fermentation rates, or morphology, and the selenium-yeast is normal in all structural and metabolic functions;
- The single chemical difference noted when comparing the two yeasts is the reduction in total sulfur in the protein fraction of about 14-20% of the standard baker's yeast; the mass balance data following selenium speciation explains the substitution of selenium for sulfur in methionine and cysteine and their anabolic or catabolic forms;
- Comparative gassing power rates, a direct yeast fermentation performance test, for the selenium-yeast and the standard baker's yeast were very similar or were equal, supporting the interpretation that the selenium-yeast has the normal complement of proteins, enzymes, and co-enzymes needed to digest the sugar at rates comparable to the normal yeast; and
- The high-selenium yeast must have the selenium content maintained at about 1325 μg/g of yeast or less to meet this performance criterion. Selenium-yeast containing selenium in place of sulfur yielding selenium content of 1400 – 2000 μg/g of yeast did diminish the gassing power rate, thereby indicating some alteration or loss of functionality in the yeast due to metabolic changes or low-level toxicity. At even higher selenium levels, such as at 2400 μg/g of yeast, the yeast fails to digest the sugar, and no fermentation is observed.

G. Intended Dietary Use

(1) Intended Food Categories in Which SelenoExcell® Will Be Used

FDA has defined several food categories in 21 CFR 170.3(n). Those categories listed below, excluding products intended for use by infants or babies and toddlers---such as infant formula or baby and toddler foods (cereals, juices, etc.)---constitute the designated food categories in which CSI intends to add SelenoExcell®, along with the anticipated use levels and maximum numbers of daily servings.

The estimated maximum number of servings per day of selected foods within each of the designated food categories is found in Table 6. It is difficult to forecast intake of specially formulated foods because statistical data on normal intake do not distinguish specially formulated foods from others in the same category. Moreover, some individuals will seek out such foods because of the specific fortification while others may choose other products because of possible extra costs resulting from fortification. These estimates attempt to reflect a consumption pattern that probably exceeds the individual consumption level.

Table 6. Proposed Food Categories for Use of SelenoExcell®

Food Category	Intended Food Products	Daily # Servings	Se Use Levels/Serving	Total Daily Se Exposure
21 CFR 170.3(n)(1)	baked products	4	5 µg	20 µg
21 CFR 170.3(n)(3)	beverages, nonalcoholic	3	5 µg	15 µg
21 CFR 170.3(n)(4)	breakfast cereals	3	5 µg	15 µg
21 CFR 170.3(n)(23)	grain products & pastas	2	5 µg	10 µg
21 CFR 170.3(n)(31)	milk products	3	5 µg	15 µg
21 CFR 170.3(n)(35)	processed fruits/fruit juices	2	5 µg	10 µg
21 CFR 170.3(n)(36)	processed vegetables/veg juices	2	5 µg	10 µg
21 CFR 170.3(n)(40)	soups & soup mixes, commercial	2	5 µg	10 µg
Total Anticipated Dietary Selenium Exposure Per Adult Per Day from Designated Food Categories:				**<100 µg**
	medical foods	20	5 µg	100 µg

In particular, it seems to be unlikely that consumers would consistently choose an average of 20 servings per day with high-selenium yeast incorporated. Nevertheless, we have chosen this scenario to ensure that potential exposure is not underestimated. This upper bound estimated daily intake, or EDI, of 100 µg of selenium from SelenoExcell® will be compared to acceptable daily intakes, or ADIs, as discussed in Section IV.

To validate the assertion that the proposed numbers of servings per food category as depicted in Table 6 are not underestimated, the USDA Continuing Survey of Food Intakes by Individuals was consulted (USDA, 1996). While the USDA food intake categories are not aligned precisely with the FDA food categories found in Table 6, useful information for comparison purposes was extracted. Considering each of the designated food categories discussed below (other than commercial soups and soup mixes), pertinent USDA food survey data were located and were found to support the view that the proposed dietary consumptions to the subject high-selenium yeast constitute an exaggerated dietary exposure.

Baked Products, Breakfast Cereals, and Grain Products & Pastas. The USDA survey reveals that the average US consumer eats 6.7 servings of grain products per day. Three FDA food categories found in Table 6---baked products, breakfast cereals, and grain products and pastas---fall under the USDA grain products category. The total number of servings proposed in Table 6 for these FDA food categories is 9, a figure that exceeds the actual consumption level reported by USDA.

Non-Alcoholic Beverages. FDA's description of non-alcoholic beverages is limited to soft drinks, coffee substitutes, and selected specialty beverages; a total of 2 daily servings was proposed in Table 6. The corresponding USDA survey reports a daily consumption of non-alcoholic beverages (excluding teas and coffee) of 342 grams or about 12.2 ounces. Using 12 ounces as the serving size for such beverages, the average consumer ingests 1.0 serving of non-alcoholic beverages per day. The Table 6 projection is 2 times greater.

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Milk Products. Milk products as described by FDA include flavored milks and milk drinks, dry milk, toppings, snack dips, spreads, weight control milk beverages, and other milk products. This food category does not include frozen dairy desserts, whole, low fat, or skim milk, or cheeses which comprise a major component of the dairy group as surveyed by USDA. The USDA survey reports that 1.5 servings of dairy per day are consumed by the average US consumer, whereas Table 6 proposes a

total of 3 servings of milk products per day for a narrower selection of dairy foods---an over estimate of more than 2.

Processed Fruits and Fruit Juices. The FDA category of processed fruits and fruit juices includes juice punches, "-ades," and juice concentrates, whereas the USDA survey also includes fresh fruits. Even with the inclusion of fresh fruits in the USDA food survey, it was reported that a total of 1.5 servings of fruit are eaten daily by the average US consumer. Table 6 allows for a total of 2 servings of the processed fruits and fruit juices, again demonstrating the absence of underestimating consumer exposures as summarized in the table.

Processed Vegetables and Vegetable Juices. As with processed fruits and fruit juices above, the FDA food category of processed vegetables and vegetable juices falls within a more broadly defined USDA food category of vegetables since the USDA survey, which reports a daily consumption of 3.3 servings per person, includes all vegetables and vegetable juices---fresh and processed. The estimated daily number of servings of processed vegetables and vegetable juices found in Table 6 is 2. Thus, the proposed consumption of processed vegetables and vegetable juices probably constitutes an exaggerated consumption level if one were to factor out the fresh vegetables and vegetable juices.

Commercial Soups and Soup Mixes. USDA survey data on likely daily exposure to commercial soups and soup mixes could not be located for a comparative analysis of consumption as suggested in Table 6. Nonetheless, the estimated consumption of 2 servings of soup on a daily basis would likely be an overestimate for the average consumer since soups do not commonly seem to be consumed on a day in and day out basis by most individuals.

Medical Foods. If those individuals on a medical foods diet were to typically consume 20 food servings of medical foods per day with the use level/serving of SelenoExcell® providing 5 µg of selenium, the total daily dietary exposure of selenium would be 100 µg---an amount that is equal to the highest amount considered to be generally recognized as safe. This estimate assumes that the medical foods would functionally replace 20 servings of traditional foods from the diets of those requiring medical foods. Because dietary management of such individuals is under the supervision of a physician, intake of SelenoExcell® would be monitored to ensure safe use.[8]

(2) Intended Technical Effect to be Achieved in Foods

SelenoExcell®, when added into the foods listed in Section II.G.(1), is to function as a nutrient supplement as defined by FDA in 21 CFR 170.3(o)(20).

It is intended that the addition of SelenoExcell® into food categories described above would facilitate avoidance of selenium deficiency to this essential micronutrient.

[8] Rayman has recently pointed out that some cases of cardiomyopathy in the West have been reported with subjects who are on intravenous nutrition that contain inadequate selenium content in their infusion solutions (Rayman, 2008). Consequently, incorporation of high-selenium yeast in selected medical foods would be desirable.

III. SCIENTIFIC EVIDENCE OF SELENOEXCELL® SAFETY

A. Introduction

In addressing selenium toxicities, Reid, et al., reference accounts of toxicities due to selenium ingestion dating to the time of Marco Polo (Reid, et al., 2004). The earlier reports of physiological manifestations of selenium in the mid-1900s focused on animal toxicities (Combs, 1997), and it was not until 1957 that the essential role of selenium in maintaining health was first documented (Hawkes, et al., 2003 & Combs, 1997). Epidemiological data that surfaced in 1969 first suggested an inverse correlation between cancer mortality and geographic distribution of selenium in forage crops (Combs, 1997). Interest in the health consequences of selenium in animals and humans accelerated since the 1970s, and we now have an extensive collection of scientific and medical information to draw upon as the topic of safety and toxicity is considered further.

An intriguing aspect of selenium ingestion, as with other essential micronutrients, is the fact that negative health consequences are associated with both selenium deficiencies and excesses, whereas there is a range of intermediate selenium ingestion levels that is deemed essential for maintaining good health.[9] Man has consumed selenium as part of the normal diet dating to antiquity because of the natural presence of selenium in various plant and animal foods. The intake levels have historically been highly variable since the natural presence of selenium in soils around the world is known to be highly variable. Hence, we note both the existence of selenium deficiencies and excesses.

CSI has compiled a foundational collection of scientific publications that address the role of selenium with several health conditions, including:

- Selenium and overall cancer;
- Selenium's effect on prostate cancer;
- Selenium and lung cancer;
- Selenium and colon cancer;
- Selenium and thyroid regulation;
- Heart disease and its association to selenium;
- Selenium and bioavailability;
- HIV; and
- Male sperm motility.

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We can see from this collection of scientific articles that selenium intake levels that are 2-4 times the US Reference Dietary Intake reduce the incidence of selected types of cancers.[10]

Schrauzer pointed out that the practice of selenium supplementation, including the use of high-selenium yeasts, increased in the mid-1970s (Schrauzer, 2001.) This trend of selenium supplementation has

[9] Section II.A summarizes the variable consumption levels of selenium from many countries around the world; note that several official governmental assessments have designated recommended daily levels intended to prevent deficiencies.

[10] In May/June, 2008, an updated scientific literature review was conducted by Dr. Mark E. Whitacre, CSI's COO and President of Operations. Whitacre, who was formally trained as a nutritional biochemist at Cornell University under the direction of Dr. G. F. Combs, Jr., has selenium-specific research credentials in addressing the health consequences of selenium ingestion in humans and animals. The updated scientific literature review augments the earlier, foundational scientific studies referred to above.

continued, and several selenium supplements are presently available in the US marketplace (Rayman, 2008). From a historical perspective, we see that selenium has been a natural part of man's diet---albeit in variable and only semi-quantified amounts (see Table 2)---and that supplementation has increasingly become part of man's diet.

The incorporation of high-selenium yeast into specific foods as the levels designated in Table 6 is intended to reduce/eliminate Se deficiencies in the US diet while also avoiding sustained dietary excesses. In other words, the proposed actions with high-selenium yeast will assist in avoiding the extremes as depicted in the U-shaped dose-response curve.

What are the clinical manifestations of selenium toxicity or selenosis? Symptoms of selenosis include: gastrointestinal upset; hair loss; dermatitis; mottled teeth; hypersalivation; white, blotchy and brittle nails; garlic breath; fatigue; irritability; changes in selected biochemical parameters; and neurological damage (National Institutes of Health, 2006, European Commission, 2000, Expert Committee, 2003, Nuttal, 2006, FDA, 2008, & EFSA, 2008).

It has been reported that a selenium intake of 250,000 μg as a single dose, or multiple doses of 27,000-31,000 μg, result in acute toxicity as demonstrated by the known selenosis symptoms (European Commission, 2000). In his review of human cases of selenium poisoning, Nuttal reported two instances of selenosis that occurred because of superpotency of selenium-containing supplements. In the first case, the tablets contained 500-1000 times the labeled amount, and in the second case, the selenium content was established to be 31,000 μg per tablet rather than the declared amount of 150 μg per tablet (Nuttal, 2006). In 2008, acute toxicity due to ingestion of superpotent dietary supplements resulted in 43 cases of human selenosis that were reported to FDA (FDA, 2008).

According to FDA regulations,[11] safety assessments that yield GRAS determinations can be based on scientific procedures or such determinations can be established through experience with common use in food prior to January 1, 1958. While we have unequivocal evidence regarding the long-term consumption of selenium by man that pre-dates 1958, this GRAS assessment relies primarily on scientific procedures and associated human and animal studies conducted over the past 40+ years. Furthermore, there exists a large body of information dealing directly with human consumption of selenium. Those studies and reviews addressing the safety and toxicity manifestations of selenium, with a particular focus on the high-selenium yeasts, SelenoExcell®, and selenomethionine, are discussed below.

B. Human Experience and Testing

(1) Summaries of Safety Reviews and Key Scientific Articles

A number of individuals and organizations, including three European groups and the US Environmental Protection Agency, have undertaken detailed evaluations of the safety of selenium as a dietary component. The published reviews have addressed safety and toxicity of selenium, including high-selenium yeast forms, based on generally available primary scientific literature. The original studies that are commonly cited in considering selenium safety in humans include the works of Yang and coworkers, Longnecker and coworkers, and Clark and coworkers (Yang, et al., 1983; Yang, et al, 1989; Yang & Zhou, 1994; Longnecker, et al., 1991; & Clark, et al., 1996).

[12] See 21 CFR 170.30(a).

European Commission. In 2000, the Scientific Committee on Food, working under the auspices of The European Commission's Health & Consumer Protection Directorate-General, issued a report that addressed the tolerable upper intake level of selenium (European Commission, 2000). This evaluation included determining the actual intake levels of selenium in their various forms for various European countries while also considering key scientific parameters that have an impact on overall safety considerations. Metabolism with bioavailability and physiological functions of selenium were addressed in the context of ascertaining daily requirements for the subject mineral. The report also acknowledged that selenium deficiency was a particular concern for many populations, including several European populations. The evaluation focused principally on adverse and toxic effects linked with selenium intake.

Acute toxicity was noted with the inorganic salts of selenium and with selenomethionine. At doses of 250,000 μg as a single dose or multiple doses of 27,000-31,000 μg, acute toxicity was noted with classical symptoms of selenosis. However, no serious cases of selenium toxicity were recorded.

Human exposures to selenium in different forms were discussed, and the results from several of the original studies have been compiled into Table 7. Selected studies will be discussed more fully below.

Daily dosages ranged from a low of 32 μg to nearly 6700 μg. More commonly, the daily dosages considered tended to fall around 200 to 600 μg. The exposures were as short as 6 weeks while others lasted for years. Evidence of selenosis or selenium intoxication was absent except for the highest exposure groups.

The following conclusions that support a determination of dietary selenium safety were offered:

- no data exist to suggest that selenium forms used in foods are carcinogenic;
- genotoxicity was detected in *in vitro* and *in vivo* systems but only at toxic doses that do not reflect nutritionally appropriate intakes; and
- no evidence was found for teratogenicity in humans (or macaque monkeys).

It was also concluded that the minimum daily dietary intake sufficient to cause selenosis is about 1200 μg. In fact, there were no clinical signs of selenosis in humans with a daily selenium intake of 850 μg. Interestingly, individuals with symptoms of selenosis when consuming 913 μg/day recovered from selenosis when their mean intakes were reduced to 819 μg/day. Studies where daily Se intakes were found to be 239 μg and 290 μg revealed no signs of toxicity.

Upon considering the accumulated information, the Commission indicated that the Lowest Observed Adverse Effect Level (LOAEL) for selenium would be 900-1000 μg/day since no clinical signs of selenosis were reported for individuals with a daily intake of 850 μg. The Commission determined the No Observed Adverse Effect Level (NOAEL) to be 850 μg based principally on the work of Yang and colleagues (Yang, et al, 1983; Yang, et al., 1989; & Yang & Zhou, 1994). The NOAEL of 850 μg/day was reduced by an uncertainty factor of 3 in yielding a Tolerable Upper Intake Level (UL) of 300 μg.

The Committee, in making this determination, reported that the 300 μg/day figure covered selenium intake from all sources of food, including supplements. This particular Se level is compatible with the findings of Clark, et al., who failed to observe any signs of selenosis over the course of a 10+ year study

with 200 μg of high-selenium yeast supplementation, along with about 100 μg of selenium coming from the diet (Clark, et al., 1996).

The Committee stated that the UL of 300 μg/day also applies to pregnant and lactating women since no data suggest that other life-stage groups have increased susceptibilities to this selenium intake. Furthermore, children were not found to be at increased risk. Consequently, the UL was adjusted downward for children in direct proportion to their body weights.

The following conclusion was offered by the Committee:

> "The Committee found sodium selenate, sodium selenite, and sodium hydrogen selenite acceptable for use in food for particular nutritional uses, but did not find other forms of selenium acceptable on the basis of current data. Therefore, the UL of this report relates only to the selenium compounds found acceptable and, in addition, to selenium naturally present in food."

The Committee did not extend its conclusion that the UL of 300 μg/day would apply to high-selenium yeasts.

Rayman, 2004. Rayman undertook a thorough review of the literature on high-selenium yeasts to assess its overall human safety. This was undertaken because of the findings by the European Committee that did not extend the conclusions of safety of selenium at a level of 300 μg/day beyond the selenate and selenite forms to the high-selenium yeast forms. The Rayman evaluation took into consideration known levels of selenium consumption, biosynthesis and metabolism of the selenium-enriched yeasts, manufacturing methods with quality control procedures, speciation of the subject yeasts, toxicity studies, and human intervention studies. Many of the studies considered are listed in Table 7.

In her review of the various studies, Rayman pointed out that the individual studies are consistent with a NOAEL of 819 μg/day, the agreed-upon figure that was embraced by the European Committee and by the Expert Group as outlined below. Other safety conclusions were offered:

- the selenium-yeast supplement is without toxic effects even after chronic dosages at 300 μg/day;
- there is no evidence that the high-selenium yeast supplementation causes a continuing rise in tissue selenium as had been hypothesized;
- selenium-yeast used at 200, 300, 400, and even 800 μg/day for lengthy periods of time (up to 12 years at the 200 μg/day dosage) were without indication of toxic effects; and
- high-selenium yeast is a safe, bioavailable form of selenium that mimics foods of selenium which is useful as a precursor for selenoprotein synthesis and as a human anticancer agent.

European Food Safety Authority, 2008. The European Food Safety Authority (EFSA) was asked to prepare a scientific opinion on the safety and bioavailability of selenium-enriched yeast as a source for selenium when added to foods and nutritional supplements for nutritional purposes for the general population. The subject safety conclusion was limited to high-selenium yeast that is produced using sodium selenite as the selenium source. This evaluation expands the safety assessment by the European Commission in 2000 that offered its finding on dietary selenium safety with its UL of 300 μg/day that did not extend to high-selenium yeast forms.

EFSA recognized that selenomethionine accounted for 60-85% of the total selenium species in the high-selenium yeast, and selenocysteine was the next most abundant species which accounted for about 2-4% of the total selenium species. It was noted that selenomethionine is readily absorbed from the gastrointestinal tract, that it is readily bioavailable, and that selenomethionine and other organoselenium species tend to be 1.5 - 2 times more bioavailable than the inorganic forms of selenium.

Repeated dietary exposure to high-selenium yeasts, as well as other dietary selenium sources, yields plasma and tissue levels of selenium that reach a steady-state level within about 2-4 months. The selenium levels do not continue to increase indefinitely, thereby mitigating concerns with selenium toxicity due to tissue accumulation of particularly high levels.

EFSA observed that the increased bioavailability of selenium from organic sources of selenium as with the high-selenium yeasts did not translate to increased toxicity for the organic selenium species. Animal test results were cited as documentation that the toxicity was found to be lower than that of inorganic selenite or selenate. This position was further supported with results from clinical studies with selenium-enriched yeast where no evidence of toxicity was observed following selenium intakes of up to 343 μg/day which extended over a period of 4 years.

The EFSA Panel reported that the highest long-term chronic dietary intakes of selenium without reported toxicity is about 800 μg/day, while noting that prolonged intakes of selenium at 1000 μg/day or higher did trigger adverse reactions. Similarly, a mean daily chronic intake of 750 μg/day did not induce selenosis.

Upon considering all of the accumulated information on high-selenium yeast, including bioavailability, metabolism, and toxicity of the subject material and selenomethionine, the Panel concluded that the high-selenium yeast when used in foods (including food supplements) for particular nutritional purposes and when consumed at levels up to 100 μg/day "does not present a safety concern." The Panel cautioned, however, that dietary augmentation with selenium at levels of 200 μg/day in addition to dietary intakes occurring naturally from foods could result in individuals exceeding the Tolerable Upper Intake Level of 300 μg/day.

Rayman, 2008 & Rayman, et al, 2008. In her ongoing evaluation into the roles of selenium on human health, Rayman has explored additional avenues to provide a more refined understanding into the complexities potentially impacting an understanding of the optimal selenium intakes. While acknowledging that both selenium deficiencies and excesses can be detrimental, the question of optimal dietary intakes between these extremes can be affected by several important variables. The number of factors that has a bearing on the effects of dietary selenium makes it difficult to ascertain what selenium levels would be of greatest value while avoiding adverse experiences.

Natural variations in dietary intake as a consequence of foods being grown in different geographic variations was recognized as a contributing factor since this influences baseline dietary selenium levels. Selenium content also varies among different foods, and the manner in which foods are cooked can also affect the selenium available from the diets. An individual's unique health status will also have a bearing on that individual's need for dietary selenium. Along with the amount of selenium ingested, the particular selenium species consumed impacts one's selenium status due to variability in bioavailabilities of different selenium species, a position echoed by others (Zeng & Combs, 2008). It was noted that selenomethionine is the predominant selenium species present in high-selenium yeast (see Table 4), and Rayman reported that it is not very toxic to cells in culture nor to animals nor to

humans, nor is there any evidence to support the allegation that organic forms of selenium are more toxic than inorganic forms (Rayman, et al., 2008).

In recognizing the complexities associated with the above, Rayman commented that care should be exercised in supplementing the diets with selenium (Rayman, 2008).

Food and Nutrition Board, 2000. Under the umbrella of the US National Academies of Science, the Institute of Medicine's Food and Nutrition Board, as part of its broader mission of providing quantitative assessments of various nutrients to benefit the health and well-being of Americans and Canadians, has undertaken its review of pertinent scientific literature of selenium to provide its recommendation for Dietary Reference Intake (DRI), and the Tolerable Upper Intake Levels (UL).

As seen in Table 3, the selenium DRI for adults is 55 μg/day and the UL was determined to be 400 μg/day.

Environmental Protection Agency, 2006. As part of its Integrated Risk Information System (IRIS), EPA evaluated the available health information on selenium. The assessment provides an oral Reference Dose (RfD) which is an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

EPA reported the LOAEL to be 1261 μg/day with the NOAEL of 853 μg/day, figures that are consistent with the conclusions by others. The RfD was calculated by applying an uncertainty factor or 3 to the NOAEL to account for sensitive individuals, yielding 5 μg/kg of body weight/day or about 300 μg/day for a 60 kg adult.

Expert Group on Vitamins and Minerals, 2003. The Department of Health in the United Kingdom, working through the Medicines and Healthcare Products Regulatory Agency, convened an expert committee of independent experts to assess the Safe Upper Levels for various vitamins and minerals. Selenium was one of the minerals considered.

Along with a summary of pertinent background information on selenium's natural occurrence in foods, its chemistry, physiological functions, and pharmacokinetic characteristics, the safety and toxicity aspects were addressed in some detail. Animal testing data will be presented in Section III.C.

Human exposures to selenium in various forms served as the principal focus of this Committee, and those individual studies that were considered by the Committee are incorporated in Table 7 and are summarized elsewhere in Section III. As was done by the European Commission, the dietary selenium exposures were reviewed in an attempt to ascertain the LOAEL and NOAEL that would serve as the basis to establish a Safe Upper Level for selenium.

Predictably, and as was the case with the European Commission, studies published by Yang and colleagues, along with Longnecker, et al., and Clark, et al., were viewed as pivotal (Yang, et al., 1989, Yang & Zhou, 1994, Longnecker, et al., 1991, & Clark, et al., 1996).

The Expert Group calculated the estimated maximum selenium intake value from food and supplements together to be 400 μg/day.

Selenosis is thought to develop when selenium intakes exceed 850 µg/day (or 14 µg/kg body weight for a 60 kg adult). Selenium supplementation at a daily level of 300 µg is not associated with overt adverse effects over a short period of time.

Marginal selenium toxicity was concluded to be present with daily intakes of 910 µg, and this figure was considered to be the LOAEL. An uncertainty factor of 2 was applied to the LOAEL to yield the NOAEL of 450 µg/day. If one considers the daily selenium intake from foods to be 100 µg, supplementing with an additional 200–250 µg falls within the calculated NOAEL of 450 µg/day. This conclusion was reinforced by the findings reported by Clark and colleagues and Longnecker and colleagues who noted no adverse effects following long-term selenium exposure at comparable levels (Clark, et al., 1996 & Longnecker, et al., 1991).

As was the case with the European Commission, the Expert Group did not identify any specific vulnerable groups.

Yang, et al., 1983. Yang and colleagues investigated endemic selenium intoxications due to high selenium in soils in China. When considering 248 individuals from five villages with a daily selenium intake of approximately 5,000 µg, 49% morbidity was detected. Classical symptoms of selenosis that affected hair, nails, and skin on feet, hands, and outer sides of the legs, forearms, and neck were observed, and neurological disturbances were particularly evident in one of the five villages. At a later stage, numbness, convulsions, paralysis, and motor impairment developed.

The daily selenium intake level with those who exhibited clinical signs of selenosis was estimated to range from 3,200-6,690 µg with an average of 4,990 µg. The afflicted residents did recover from selenosis as soon as the diets were modified with a downward adjustment in selenium to 240-1510 µg with a mean intake of 750 µg. The principal form of selenium consumed from the diet of rice and maize was established to be selenomethionine.

Yang, et al., 1989. Yang and colleagues continued their investigations of selenium ingestion in different regions of China, considering areas to be "low," "medium," or "high." Average daily selenium intakes for adult males were found to be 70, 195, and 1438 µg, respectively; the average daily selenium intakes for adult females were 62, 198, and 1288 µg, respectively. A total of 349 adults was part of the study. Clinical selenosis was observed in the high selenium area, but no clinical signs were observed when the daily selenium intake was 853 µg or less. Five individuals who had long, persistent clinical signs of selenosis were found to have a daily intake ranging from 913-1907 µg with a mean of 1260 µg. A selenium intake level of 910 µg/day was determined to be a level of marginal selenium toxicity. No specific neurological symptoms were found and there was no evidence of birth defects in humans.

Yang & Zhou, 1994. Yang and Zhou performed a follow-up study on the 5 selenosis subjects cited in Yang, et al., 1989. Incorporating dietary changes with somewhat limited selenium content, with a daily intake of 800 µg, yielded a loss of clinical signs of selenosis. The authors determined that a daily selenium intake of 800 µg represented a NOAEL, and 400 µg/day was recommended as the maximum safe daily dietary intake.

Longnecker, et al., 1991. A total of 142 subjects residing in regions of the US where selenium levels are naturally high were followed over a 2-year period of time. The daily selenium dietary intake was assessed, and subjects were monitored for adverse health effects. Selenium measurements were taken from whole blood, serum, urine, and toenails over the course of the study.

The average selenium intake was 239 µg/day, with the range spanning 68-724 µg/day. Half of the subjects had intake levels above 200 µg/day, and 12 individuals had intake levels exceeding 400 µg/day. Although it was noted that the alanine aminotranferase levels in serum were elevated, it was concluded that the values were within the reference range and were clinically insignificant. There was, however, an increased sense of lethargy associated with increased selenium values. It was concluded that no physical characteristics of selenium toxicity had manifested, nor were there any other significant effects attributed to elevated selenium intakes.

Clark, et al., 1996. Clark and colleagues conducted a randomized, double blind, placebo-controlled study on the effects of selenium supplementation on the prevention of skin cancer. 1312 patients with a history of basal cell or squamous cell carcinoma were treated with 200 µg of SelenoExcell® or placebo for up to 10 years (mean of 4.5 years). Patients were assessed semiannually for known signs of frank selenosis---no indications of dermatological or other signs of selenium toxicity were detected. A total of 35 patients, including 14 from the control group, did experience gastrointestinal upset which prompted them to withdraw from the study.

While the selenium supplementation did not have an effect in reducing non-melanoma skin cancer, the study did reveal a 50% reduction in total cancer mortality, 37% lower total cancer incidence, a 63% reduction in prostate cancer, a 58% reduction in colon cancer, and 46% fewer cases of lung cancer.

The total dietary intake of selenium from supplementation (200 µg), along with the contributions from food (90 µg), totaled 290 µg/day. Also see Clark, et al., 1998.

(2) On-Going Selenium Clinical Trials

In addition to the selenium intake studies referenced above, other selenium supplementation investigations continue or are under development (Nutraingredients, 2006). Several studies focus on selenium prevention or inhibition of prostate cancer, and others address other forms, such as liver, colorectal, esophageal, breast, and lung cancer. Still other investigations seek information about a possible preventive role for selenium with Alzheimer's disease, non-cancerous liver disease, and improved immune function.

A multi-center, National Cancer Institute-sponsored program, the SELECT program (Selenium and Vitamin E Cancer Prevention Trial) involving many thousands of subjects will continue through 2013 (Nutraingredients, 2006). Another multi-center cancer clinical trial is underway under the auspices of the Eastern Cooperative Oncology Group (ECOG) with support from NCI (ECOG, 2007). In this investigation, the potential role of orally administered selenium in preventing the development of a second primary lung tumor in patients who previously underwent surgery to remove stage 1 non-small cell lung cancer is under evaluation.

Table 7. Summary of Selected Human Testing Studies with SelenoExcell®, Other High-Selenium Yeast Products or Selenomethionine[12]

Reference	No. of Subjects	Estimated Daily Dosage (µg)	Duration	Comments
(a)	2	350 & 600	18 m	Marginal hematological changes; borderline increase in serum alanine amino transferase
(a)	4 M per group	32.4, 206, & 388	?	No adverse effects reported
(a)	6 M per group	200	6 w	No adverse effects reported
(a)	32 F	450– 500	3 m	Half subjects experienced depression & extreme tiredness following termination of study
(a)	125 F/lactating	90-350; 170-500; or 250-980	?	No evidence of selenosis
(b)	248	3200–6690 (4990 average)	long-term (?)	Selenium intoxication in 49% of subjects; observed skin, hair, nail, abnormalities & neurological & motor disturbances; exposures to 750 µg did not induce adverse effects; selenosis reversed with diet changes.
(c)	~400	M – 70, 195, & 1438; F – 62, 198, & 1288	long-term (?)	Marginal selenium toxicity observed at 910 µg/day but not at a daily intake levels of 853 µg or less
(d)	5	819	long-term (?)	Symptoms of overt signs of selenosis at 1270 µg/day disappeared after change in diet to 819 µg/day; NOAEL of 800 µg/day
(e)	142	68-724 (239 average)	2 y	12 subjects daily exposure exceeded 400 µg; no clinically significant observations; increased lethargy noted in some subjects
(f)	1312	290	Up to 10+ y (4.5 ave)	No clinical signs of selenium toxicity based on semiannual exams; limited patient withdrawals due to GI tract distress
(g)	226	200	4 y	Hepatitis surface-antigen positive patients; no side effects noted
(g)	500	140, 240, & 340	2 y 8 m	UK PRECISE program; no signs of selenium toxicity reported as of Jan 2003; some reports of GI discomfort
(g)	500	143, 243, & 343	4 y 8 m	Danish PRECISE program; no evidence of selenium toxicity reported as of Aug 2003; some reports of GI discomfort
(g)	Small group	200-600	3-8 m	Rheumatoid arthritis patients experience pain & morning stiffness relief without adverse effects
(g)	? lactating mothers	200	3 m	Doubling of infant intake of Se without reports of adverse effects; Se levels remain 4-5 times below upper limit for infants from Food and Nutrition Board
(g)	22	100 supplement	6 m	Elderly subjects experienced improvement in age-related decline in immune response
(g)	186	200 supplement	2 y (?)	Beneficial treatment adjuvant for HIV-positive subjects without reported adverse effects
(h)	?	200	2 y	Liver cancer preventive study using high-Se yeast without adverse effects noted
(h)	3698	50 + vitamin E + β-carotene	8 y	Modest protection against stomach cancer
(h)	3698	50 + vitamin E + β-carotene	8 y	Modest protection against stomach cancer
(h)	29,584	50 + vitamin E + β-carotene	2+ y	Cancer prevention study determined that total mortality & cancer mortality lowered when using the selenium combination
(h)	298	100 followed by 50 (combination formulation)	6 m + 6 m	Frequency of micronuclei & DNA adducts reduced by 95% & 72% in different study groups; no adverse effects noted
(h)	304	200 (combination formulation)	5 y	Reduced incidence of metachronous adenomas in large bowel by half

(a) European Commission, 2000 & references found therein.
(b) Yang, et al., 1983.

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[12] While two studies reported adverse effects (depression and tiredness and age-related decline in immune response), these observations were not reported in other studies and are unlikely to be related to intake of the test article.

(c) Yang, et al., 1989.
(d) Yang & Zhou, 1994.
(e) Longnecker, et al., 1991.
(f) Clark, et al., 1996.
(g) Rayman, 2004 & references found therein
(h) Whanger, 2004 & references found therein.

The conclusions drawn from the clinical trials to date are compelling in that elevated levels of dietary selenium that are 2-4 times (or more) the US DRI level of 55 μg per day per adult impart health benefits, such as cancer prevention, inhibition of genetic damage, or enhanced immune system performance (Whanger, 2004). To expand the current state of knowledge and gain greater depth of understanding, scientists and health care professionals in the US and around the world are actively engaged in the design and execution of clinical trials involving selenium supplementation.

Long-term selenium supplement investigations continue at the Arizona Cancer Center, University of Arizona, the Cancer Institute at the Chinese Academy of Medical Science in Beijing, in the UK as part of the PRECISE (Prevention of Cancer by Intervention With Selenium) program, and in Denmark as part of the PRECISE program. The studies that are in process through the PRECISE program are considered to be pilot studies with the hope to expand the studies to 14,500 subjects covering 7 years.

Several prostate cancer chemoprevention studies are in process at the University of Arizona as a result of the profound results reported by Clark, et al. (Clark, et al., 1996 & Clark, et al., 1998). According to Marshall, the study designs capture a continuum ranging from short-term effects on healthy and cancerous prostatic tissues in men with diagnosed cancer, to long-term effects on healthy and premalignant tissue in men with high-grade prostatic intraepithelial neoplasia, to long-term effects on healthy tissue in high-risk men with negative biopsy, to long-term effects on cancerous tissue in men with frank cancer (Marshall, 2001). Marshall has described the four studies as follows:

1. Negative Biopsy Trial – selenium treatment of men who received negative results from prostate biopsies;
2. Selenium treatment of men with high-grade prostatic intraepithelial neoplasia;
3. Preprostatectomy Trial - selenium treatment of men with localized prostate cancer before prostatectomy; and
4. Watchful Waiting Trial - use of selenium as a chemotherapeutic agent among men with confirmed prostate cancer.

Experimental design details are contained within Table 8.

Stratton, et al., 2003a. The results of the Clark study were remarkable in the suggestion that the incidence of certain types of cancer, including prostate cancer, can be diminished as a result of high-selenium yeast supplementation at the total daily exposure of 300 μg (food and supplement) (Clark, et al., 1996). There is a need to confirm these findings to enable a more aggressive use of selenized yeast in cancer prevention, and the subject investigation, along with the studies reported in the following two summaries, are intended to do just that. 700 men considered to be at high risk for prostate cancer, but who have had a negative prostate biopsy, were administered high-selenium yeast supplements daily at the level of 200 or 400 μg. Can these selenium doses decrease the incidence of prostate cancer in high risk men? The study design and initial results are described. In addition, the subjects are being monitored for specific serum markers that might indicate biochemical progression of prostate cancer more effectively. Measuring both alkaline phosphatase and chromagranin A levels was built into this study.

As of June, 2003, a total of 514 men were enrolled in the study. No new safety concerns were raised during the early stages of the study.

Stratton, et al., 2003b. As was the case with the above-noted Negative Biopsy Trial, the Watchful Waiting Trial is an extension of the work reported by Clark, et al. (Clark, et al., 1996). The watchful waiting approach is a more desirable option for some men who are diagnosed with prostate cancer since selenized yeast supplementation could delay the necessity for surgery, radiation treatment, or some other invasive treatment modalities.

During the watchful waiting period, monitoring key biomarkers for progression of prostate cancer would be particularly valuable so health care providers can more confidently distinguish between slow- and fast-growing prostate cancer. The study design for the Watchful Waiting Trial includes administration on a daily basis of either 200 or 800 μg high-selenium yeast to 264 men with the trial to last at least 4 years and possibly up to 5 years. Over the course of the study, certain biomarkers, such as alkaline phosphatase and chromagranin A, along with other potential biomarkers, will be measured to provide insights into the progression of prostate cancer.

As of June, 2003, a total of 191 subjects of the targeted 264 had been recruited. No safety concerns had been raised during the early stages of this trial.

Reid, et al., 2004. As part of the Watchful Waiting Trial described above by Marshall, it was hoped to gain insights into the toxicity of selenium when administered in chemoprevention in appreciably increased dosages that exceeded the NOAEL of 400 μg dosage. 24 men with biopsy-proven prostate cancer were given either 1600 or 3200 μg/day of high-selenium yeast. Selenium levels in plasma and symptoms of selenium toxicity were assessed periodically over the course of 12 months. Liver and kidney function tests and hematology were measured at 6-month intervals. The 3200 μg/day group reported more selenium-related side effects, but blood chemistry and hematology results were in normal limits for both dosage groups. No obvious selenium-related serious toxicities were observed.

This trial has presented the highest test doses of high-selenium yeast to humans over a sustained period of time, and no serious toxicities were revealed. While these findings do not establish the safety of long-term high-dose organic selenium supplementation in the general population, the observed toxicity profiles suggest that doses greater than 400 μg/day can be given in controlled situations for extended time periods without serious toxicity.

Reid, et al., 2006 & Reid, et al, 2008. Reid and co-workers extended their investigation into the health effects of selenium supplementation through the Nutritional Prevention of Cancer trial by focusing the association between selenium supplementation with daily exposure of 200 μg of selenized yeast and prevalent and incident colorectal adenomas and colorectal cancer. While reporting favorable results with diminished cancers from this level of supplementation, there were no indications of adverse effects (Reid, et al., 2006). An additional substudy involved administering the selenized yeast at a daily exposure of 400 μg, but no increased effect in diminished lung, colon, prostate, or total cancers was observed (Reid, et al., 2008).

(3) Effects of Long-Term Selenium Supplementation and Type 2 Diabetes 000030

Stranges and coworkers (Stranges, et al., 2007) examined the effects of long-term selenium supplementation in humans on the incidence of type 2 diabetes by relying on a secondary analysis of

information from a randomized, double blind, placebo-controlled clinical trial. Yeast-bound selenium was orally administered to 1312 participants at levels up to 200 μg per day for up to 7.7 years. Despite noting limitations in the study with secondary effects, self-reporting of the type 2 diabetes diagnoses, and reliance on a limited subpopulation, the authors concluded that "selenium supplementation does not seem to prevent type 2 diabetes, and it may increase risk for the disease."

These findings prompted editorial comments from Bleys and colleagues (Bleys, et al., 2007) who addressed the need for high quality prospective studies and randomized trials to investigate the effects of dietary selenium and selenium supplementation on the incidence of diabetes. They embraced a cautious approach regarding dietary supplementation with selenium in that routine supplementation should be avoided until such studies were conducted.

Dr. G. F. Combs, Jr, Director of USDA's Grand Forks Human Nutrition Research Center and co-author of the Stranges article cited above, has continued to investigate the potential linkage between selenium supplementation and the onset of type 2 diabetes. Under his direction, a year-long intervention study was conducted to characterize the dose response relationship between selenium status and diabetes risk.

The results of this investigation have been published as an abstract (Combs, et al., 2008 and see Appendix C) wherein the authors report that **these results do not support a relationship between selenium status and diabetes risk**. Appendix C. also contains a letter authored by Dr. Combs that concludes that their study results "do not indicate a relationship of Se stratus and diabetes risk. While we recognize the inferential limitations inherent in a small study such as this, it is also the case that these are the most robust data relevant to the question of whether selenium status may be related to diabetes risk. Thus, it is our plan to continue to address this hypothesis in further studies; but for the moment, I consider the supporting evidence very weak."

(4) ADME and Bioavailability

The metabolic conversions of the different selenium forms have been well-studied and are summarized by several research groups. Cereal and forage crops, as well as *Saccharomyces cerivisiae,* absorb inorganic selenium which is converted into selenomethionine which, in turn, is subsequently incorporated into protein. Selenomethionine then undergoes other metabolic conversions. Excess Se is detoxified by successive methylation of H_2Se to yield methyl selenol (CH_3SeOH), dimethyl selenide ($(CH_3)_2Se$) and trimethylselenonium ion ($(CH_3)_3Se^+$; the latter two species are excreted in the breath and urine (Rayman, 2004, Whanger, 2004, & Infante, et al., 2005).

It is also known that the organoselenium forms of selenium, especially selenomethionine, are more bioavailable than the inorganic selenium salts (Burk, et al, 2006). Rayman reports that organic forms of selenium are absorbed to the extent of about 80%, although the bioavailability is affected by the nutrient status of individuals, the manufacturing process for the subject organoselenium form, and the presence of other dietary components, including sulfur. Nonetheless, it was concluded that human supplementation studies indicate that the selenium from high-selenium yeasts is more bioavailable than inorganic selenium (Rayman, 2004).

Table 8. Summary of Expanded Clinical Studies with SelenoExcell® and Other High-Selenium Yeast Products

Reference	No. of Subjects	Est. Daily Dosage (µg)	Duration	Comments
(a), (b)	700 M	200 or 400	57 m	Negative Biopsy Trial; intend to assess prostate cancer status of individuals with negative prostate cancer biopsies; 514 subjects were randomized to trial as of Jun 2003.
(a)	470	200	3+ y	Intend to assess prostate cancer status of men with high-grade prostatic intraepithelial neoplasia following selenomethionine supplementation.
(a)	110	200 or 400	6-8 w	Preprostatectomy Trial; evaluation during the time interval between biopsy & prostatectomy; 55 subjects recruited to trial in 2001.
(a), (c), (d)	264	200, 800, 1600 or 3200	4 - 5 y	Watchful Waiting; seeking pharmacological data from selenium exposures, along with chemotherapeutic outcomes 191 subjects recruited as of Jun 2003.

(a) Marshall, 2001.
(b) Stratton, et al., 2003a.
(c) Stratton, et al., 2003b.
(d) Reid, et al., 2004.

The Expert Group on Vitamins and Minerals reported that selenium compounds are readily absorbed from the small intestine, but the extent of absorption varies, depending on the nature of the specific compound. Selenium was found to be widely distributed throughout the body, and its presence has been detected in breast milk. Selenium levels are slightly higher in the liver and kidneys than in other tissues. It was also noted by Hawkes, et al., that the whole body retention of selenium is about 15 mg with 5 mg being retained in muscle tissue (Hawkes, et al, 2003). Selenium is largely excreted in the urine, with volatile metabolites being excreted in the breath. Some fecal excretion of selenium occurs following chronic Se administration (Expert Group, 2003).

In addressing the ADME and bioavailability topics for high-selenium yeast, EFSA acknowledged that organoselenium compounds such as selenomethionine have been documented as exhibiting enhanced bioavailability from both animal and human experience. The bioavailability of selenomethionine is more than 90 percent which is nearly twice that of selenium from sodium selenite. Furthermore, repeated and sustained dietary exposure to selenium-rich sources such as the yeast yield a steady-state within 2-4 months. Plasma and other tissues did not continue to accumulate the selenium (European Food Safety Authority, 2008).

(5) Genotoxicity

The results of *in vitro* mutagenicity tests were declared to be inconsistent, and selenium compounds are largely negative in the available *in vivo* mutagenicity tests. An increase in chromosomal aberrations in hamster bone marrow was reported, but this occurred only at lethal doses with sodium selenite (Expert Group, 2003). No genotoxicity was found to occur at nutritionally adequate intakes of selenium (Expert Group, 2003).

An extract of high-selenium yeast, determined to be ~98% selenomethionine, yielded negative testing results from the Ames test, the chromosomal aberrations in human lymphocytes *in vitro*, and the micronucleus test in mouse bone marrow (European Food Safety Authority, 2008).

(6) Reproductive Effects

Adverse effects have been reported on the reproductive systems of various animals including birds, fish, sheep, pigs, and hamsters at maternally toxic doses or under conditions of nutritional deprivation. High-selenium yeast and sodium selenate had been administered to breeding and pregnant female pigs, and the individual pig birth weights were unaffected. Similarly, litter size, number of pig deaths, and pig and litter weights at specified days postpartum were unaffected by the ingested selenium. No adverse reproductive findings were observed to occur with primates. Macaque monkeys were fed selenomethionine at a dose of 300 μg/kg of body weight/day during organogenesis with no signs of terata. There have been no reports of reproductive toxicity effects in humans even in high selenium intake regions in China (European Commission, 2000, Expert Group, 2003, & European Food Safety Authority, 2008).

(7) Allergenicity

Rayman cautions that some individuals may be allergic to the yeast that is used in the production of high-selenium yeasts. No data or reports of allergic reactions were provided (Rayman, 2004). This caution prompted a review of the scientific literature to ascertain whether or not allergic reactions were known to occur as a consequence of ingestion of *Saccharomyces cerivisiae* or the selenized yeast. The Pub Med search specifically utilized the following selection of keywords and phrases: (1) selenized yeast allergenicity; (2) *Saccharomyces cerivisiae* allergenicity; (3) *Saccharomyces cerivisiae* yeast allergenicity; (4) selenomethionine allergenicity; (5) organoselenium allergenicity; (6) selenium allergenicity; and (7) sodium selenite allergenicity.

There were no reports of allergenicity associated with the yeast forms either with or without selenium. A single report was located that highlighted inconsistent conclusions about reported benefits of administering selenium to prevent asthma compared with the possible role of selenium in influencing immune responses that could trigger or exacerbate allergic asthma (Hoffmann, 2008). Hoffmann reported that his study used a mouse model of allergic airway inflammation, and it failed to establish that Se intake affected the development of allergic asthma in a simple dose-response manner. While questions were raised about the applicability of the mouse model to humans, the author encouraged caution when considering dietary intake of selenium in concert with allergic asthma because of the complexities of the disease.

To extend the investigation further into possible allergenicity due to the high-selenium yeast, CSI's Dr. Mark Whitacre contacted key organizations shown below that are intimately involved with food allergies. Reprsentatives at these organizations were specifically asked about their awareness of food allergies linked with *Saccharomyces cerivisiae*, and none had any knowledge of such food allergies.

American Academy of Allergy, Asthma and Immunology (see www.aaaai.org)
The Food Allergy Network (see www.foodallergy.org)
American Academy of Pediatrics – Section of Allergy and Immunology (see www.aap.org)
American College of Allergy, Asthma and Immunology (see www.acaai.org)

In its recently issued opinion on the safety of selenium-enriched yeast as a source of dietary selenium, EFSA's Panel addressed the topic of allergenicity. The Panel reported that the actual amount of selenoyeast ingested on a daily basis would be small (up to 200 mg per day) and the cellular

constituents of the yeast are anticipated to be endogenous in the human body. The Panel concluded that **the food uses of the selenium-enriched yeast were unlikely to present an allergenic risk to consumers**. Furthermore, individuals with yeast sensitivity would be alerted to the presence of yeast protein via product labeling (European Food Safety Authority, 2008).

Despite the precaution expressed by Professor Rayman on possible allergic reactions linked to yeast ingestion and the possible role of selenium in triggering allergic asthma, no firm evidence of probable allergenicity associated with ingestion of *Saccharomyces cerivisiae* with or without the incorporation of selenium was found.

While it is unlikely that human ingestion of *Saccharomyces cerivisiae* with or without the incorporation of selenium would trigger allergic reactions, an addition safeguard exists, as was noted by the EFSA Panel, with ingredient labeling requirements. The incorporation of high-selenium yeast into formulated foods requires the proper finished product ingredient labeling, and such labeling of high-selenium yeast would provide those consumers concerned about yeast food allergies with sufficient information to avoid those foods. Appropriate food product labeling has become a standard and appropriate regulatory practice with food allergy concerns to assist consumers with their food decision-making even with those foods (e.g., fish, shellfish, tree nuts, milk, eggs, and peanuts) that have been well-documented to be food allergens. Consequently, allergenicity triggered by high-selenium yeast addition to foods does not constitute a safety concern.

C. Animal Testing

(1) Background Considerations

Substantial human experience and clinical testing studies that have been generated over the past four decades and as detailed in Section III B address the broad health effects pertaining to selenocompounds, and this information provides a firm basis for ascertaining what levels of different selenocompounds are safe. Furthermore, extensive animal testing on selenium compounds provides more insights into the overall safety considerations for the various forms of selenium, especially those selenocompounds that are found in foods and can be utilized for nutrient purposes.

Expert international groups have reviewed animal testing results for selenium as possible indicators of toxic manifestations in humans and have offered the following summary statements: (1) A daily dose of a soluble selenium compound of 500 μg/kg body weight was not associated with any adverse effects (Expert Group, 2003); and (2) Chronic exposures in animals at levels in excess of 0.03-0.4 mg/kg bw resulted in reduced growth rates and diminished weight gain, along with liver changes, anemia, pancreatic enlargement, and, in some cases with domestic animals, neurotoxicity (European Commission, 2000). Regarding neurotoxicity, exposures to large doses of selenium over extended periods of time result in neurological dysfunction as observed with impaired vision, ataxia, and disorientation. Of historical significance, selenium toxicity was observed in grazing livestock that fed on selenium-accumulating plants and then developed "blind staggers" (Environmental Protection Agency, 2006).

Rather comprehensive accounts of individual selenium animal studies can be found in the EPA safety assessment of selenium (Environmental Protection Agency, 2006). In addition, the Agency for Toxic Substances and Disease Registry (ATSDR) compiled a thorough review of selenium toxicity that includes a detailed accounting of animal testing results (ATSDR, 2003). In it, ATSDR considered

observed health effects in the following areas: (1) respiratory effects; (2) cardiovascular effects; (3) gastrointestinal effects; (4) hematological effects; (5) musculoskeletal effects; (6) hepatic effects; (7) renal effects; (8) endocrine effects; (9) ocular effects; (10) body weight effects; (11) immunological and lymphoreticular effects; (12) neurological effects; (13) reproductive effects; (14) developmental effects; and (15) cancer.

Reviews of the type cited above considered numerous selenocompounds, including elemental selenium, inorganic selenium, and organic selenium that includes selenoamino acids and high-selenium yeast. Particular attention was paid to studies utilizing high-selenium yeast, selenomethionine, and other organic forms of selenium. The findings did not suggest the likelihood of adverse effects for humans who would be consuming high-selenium yeast as a component in foods at levels of 100 μg Se/day as proposed by CSI.

Rayman reported that inorganic forms of selenium are more acutely toxic than selenized yeasts and the organic forms. She states: "The LD_{50} for Se-yeast is 37.3 mg/kg compared with 12.7 mg/kg for sodium selenite, demonstrating that Se-yeast is considerably less acutely toxic than sodium selenite." In further support of this conclusion, an 8-week feeding study using two different selenium forms, selenized yeast compared with selenite, at identical dietary concentrations of 16 μg Se/g of diet revealed severe hepatoxicity, cardiotoxicity, and splenomegaly in the selenite study group and no such observations with the selenized yeast (Rayman, 2004).

Rayman and colleagues expanded their consideration of comparative toxicities of selenocompounds by noting that acute and chronic animal studies provide the basis for the conclusion that inorganic forms of selenium are more toxic than organic forms, and that selenite is more toxic than selenate. A comparative rat study revealed lesser toxicity for selenium-yeast compared with selenite and that selenium-yeast exhibited less toxicity (i.e., longer survival times) than selenomethionine based on a two-week feeding study at 30 μg Se/g of diet in mallard ducklings (Rayman, et al., 2008).

The determination that organic forms of selenium exhibit lower toxicities than inorganic forms was acknowledged by EFSA in their 2008 safety opinion (European Food Safety Authority, 2008) and is further reinforced with the report that diphenyl diselenide exhibited low toxicity in an 8-month feeding study with rabbits (de Bem, et al., 2007).

The toxicity/mutagenicity potential of sodium selenite, selenomethionine, and selenomethylselenocysteine were all studied with the budding yeast *Saccharomyces cerivisiae* model system to establish potential for inducing cellular DNA damage. Only sodium selenite was observed to induce any significant toxic effects in the yeast (Letavayova, et al., 2008).

(2) Cancer Studies

000035

The earliest reports suggested that rats fed seleniferous wheat developed hepatic tumors, but the study was subsequently criticized as being scientifically inadequate (ATSDR, 2003 & Environmental Protection Agency, 2006). The only studies reporting animal carcinogenesis involved administration of selenium selenide and selenium diethyldithiocarbamate, neither of which are found in food, nor would they be nutrients. Experimental data do not indicate that inorganic or organic selenium compounds that are relevant to food and nutrition are carcinogenic (European Commission, 2000). The topic of animal carcinogenicity has triggered substantial research, with interest stemming from selenium as a causative agent and its functioning as an anticarcinogenic agent. Whanger reports that more than 100 small animal trials have been conducted since 1949 in exploring the relationship of tumor incidence and

selenium intake. The prevailing initial view was that selenium was cancer-causing, but the availability of more studies, especially since 1957 when the essential nature of selenium in humans was established, offset this conclusion. Two-thirds of the selenium animal studies conducted over the years illustrate a significant reduction in tumor incidence (Whanger, 2004). Rayman also stated that selenized yeast was found to be effective in reducing tumor yield in the animal model for breast cancer when tumor formation was induced by methylnitrosourea administration (Rayman, 2004).

An *in vivo* canine model system has been used to assess DNA damage in prostate cells and in peripheral blood lymphocytes using selenomethionine and selenized-yeast as dietary supplements. Test materials were administered as a supplement to the diets of dogs over seven months at doses up to 6 μg Se/kg bw. Dogs and humans are the only species in which prostate cancer occurs spontaneously with appreciable frequency, so the availability of a reliable canine model imparts appreciable benefit in studying prostate cancer in humans. The authors concluded that dietary selenium supplementation decreased DNA damage within the aging canines (Waters, et al., 2003 & Waters, et al., 2005).

The International Agency for Research on Cancer concluded that there was no suggestion that selenium is carcinogenic in humans based on its evaluation of the scientific literature pertaining to selenocompounds and carcinogenesis in animals and in humans (ATSDR, 2003). No specific carcinogenic studies have been conducted on high-selenium yeast or selenomethionine (European Food Safety Authority, 2008).

(3) Reproductive and Teratogenic Effects

It was reported that high selenium exposures adversely affect the female estrous cycle and the sperm concentration and quality in males. Multi-generation studies suggest that elevated selenium exposures reduce post-natal survival and weights of offspring (Expert Group, 2003). As noted in Section III.B, selenite, selenate, selenocysteine, and selenomethionine are considered to be teratogenic in birds, fish, sheep, pigs, and hamsters. However, long-tailed macaques did not show any signs of teratogenicity when fed selenomethionine at dosages of 25, 150, and 300 μg/kg bw during organogenesis (European Commission, 2000, & Expert Group, 2003), and the dose of 25 μg/kg bw was considered to be a NOAEL (European Food Safety Authority, 2008). It was also stated that positive reproductive effects in rodents due to selenium compounds are commonly linked with overt maternal poisoning and nutritional deprivation (European Commission, 2000).

Kim and Mahon reported that feeding high sodium selenite and selenium-enriched yeast at levels of 7 - 10 ppm in the diets of gilts triggered selenotic effects that included hoof separation and alopecia and diminished reproductive performance and lactation; these adverse effects were not observed at lower dietary selenium levels (Kim & Mahan, 2001). However, the addition of high-selenium yeast to the feed of female pigs from 60 days prior to breeding until weaning was subsequently reported to yield no adverse effects on reproductive performance or growth (ATSDR, 2003).

Additional studies have been performed on the effects of selenium compounds on sheep. Davis and co-workers investigated the ability of range-type ewes to tolerate dietary sodium selenite during gestation and lactation. Based on a 72-week study with daily dietary sodium selenite levels ranging from 0.2 to 20 mg/kg bw, there were no indications of selenium toxicity at any levels tested, leading the authors to conclude that there were no clinical signs of selenium toxicosis when administered at a daily dose up to 20 mg/kg bw and that such an exposure was well tolerated by the animals (Davis, et al., 2006).

Neville and colleagues examined the effects of dietary selenium from high-selenium wheat and from sodium selenate on pregnant ewe lambs. The animals received daily doses ranging from 2.5 μg Se/kg bw to 375 μg Se/kg bw from day 50 of gestation through day 134 of gestation, and no indications of toxicity were observed in any treatment groups (Neville, et al., 2008).

No studies were located that linked teratogenic effects or other adverse health effects on human reproduction to oral exposure to selenocompounds (ATSDR, 2003).

IV. EXPERT PANEL ASSESSMENT AND DISCUSSION OF COMPOSITE SAFETY INFORMATION FOR SELENOEXCELL® GRAS STATUS

FDA defines "safe" or "safety" as it applies to food ingredients as

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[13]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."[14]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[15]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and

- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

Both the scientific elements and the common knowledge elements that FDA has identified as essential components of valid GRAS determinations have been met. Considering the composite information for the intended food uses of selenium in all forms, and by relying extensively upon human experience and testing as complemented by animal testing---i.e., scientific procedures---we have concluded that SelenoExcell® is safe within the food use limitations as specified elsewhere[16] and therefore qualifies for GRAS status.

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[13] See 21 CFR 170.3(i).

[14] See 21 CFR 170.30(a).

[15] See *Federal Register* 62 April 17, 1997, 18937; or http://www.cfsan.fda.gov/~lrd/fr970417.html.

[16] It is intuitively obvious that selenium has historically been consumed as part of the human diet since we now recognize the essential nutrient status of selenium in humans, plus we know that selenium is a natural constituent of numerous commonly consumed foods across the globe. However, we do not have firm historical information on the specific selenocompounds consumed and the levels at which they have been ingested over time. Hence, historical usage considerations are not part of the subject GRAS evaluation.

There is a substantial chorus of concurrence from international experts who acknowledge the essentiality of selenium as a micronutrient in humans at levels that do not approach toxic levels that have been well established by several human studies. In fact, the works of Longnecker, et al., Clark, et al, and Yang, et al., are all cited repeated without challenge to the validity of their findings. The levels of ingested selenium that have been identified as imparting adverse effects, as presented in Section III. and as discussed below, are typically in the 750-800 μg/day range. International experts, including the most recent scientific opinion reported in July 2008 by EFSA when addressing the safety of selenium-enriched yeast, have utilized safety factors to provide ADIs or ULs of 300-400 μg.

Utilizing the generally agreed-upon safe dietary exposure of 300-400 μg/day to provide the proposed level of addition of SelenoExcell® into foods as detailed in Table 6 provides an additional margin of safety. The upper bound total estimated daily intake (EDI) for adults was calculated to be 100 μg as selenium, a dietary exposure for selenium-enriched yeast when added to foods that EFSA has validated as presenting no safety concerns. The EDI is compared to ADIs and ULs calculated from LOAELs and NOAELs as extracted from the public literature---both original, or primary, literature and review articles and expert opinions, or secondary literature, as needed to meet the "common knowledge element"---in completing the GRAS assessment for SelenoExcell®. This, when added to typical levels of Se intake, is well within accepted limits to establish its safety under the intended use conditions.

The accumulated animal toxicity investigations on organoselenium, high-selenium yeast, and selenomethionine present no indications that would offset the above safety conclusions derived from human testing and experience. It is recognized that the assessment of selenium safety and toxicity extends to various other chemical forms of selenium, and these investigations were considered by several knowledgeable bodies and other experts, as summarized in Sections III.B. and III.C. and by this Expert Panel. To be sure, it has been well-established that inorganic forms of selenium are less bioavailable and exhibit increased toxicity when compared to the organoselenium forms, including SelenoExcell®.

As noted above, the central consideration in concluding that high-selenium yeast is GRAS when incorporated into selected at foods with daily dietary exposures from the addition of foods at levels up to 100 μg was the reliance on the LOAELs and NOAELs from which safe upper limits of daily selenium exposure for humans were derived by multiple organizations and well-qualified scientists. The general convergence of scientific opinion, which strongly supports the assertion that scientific consensus exists for the subject GRAS determination, is embodied in the following summary statements:

- The Food and Nutrition Board determined that its Tolerable Upper Intake Level, i.e., the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population, was 400 μg (Food and Nutrition Board, 2000).

- The European Commission established its LOAEL to fall within the range of 900-1000 μg, and they designated its NOAEL to be 850 μg. Applying an uncertainty factor of 3 yields a Tolerable Upper Intake Level of 300 μg (European Commission, 2000).

- Rayman reviewed the safety literature on selenized yeasts and extracted a NOAEL of 819 μg. It was also noted that selenized yeasts were safely consumed at various levels up to 800 μg per day for extended periods of time, without toxic manifestations. It was concluded that the Tolerable Upper Intake Level of 300 μg/day was conservative and should also apply to selenized yeasts, such as SelenoExcell® (Rayman, 2004).

- EPA reported its NOAEL at be 853 μg/day, from which it applied an uncertainty factor of 3 to establish its oral RfD of 300 μg/day---an exposure over the course of a lifetime that is likely to be without appreciable risk (Environmental Protection Agency, 2006).

- Combs, who worked as part of the Clark research team, contends that the EPA RfD should more correctly be 600 μg/day for men and 475 μg/day for women. He supported his position by noting that selenium supplementation trials using dosages of 400 μg (for a total daily selenium exposure of 500 μg when considering the selenium contributions from the diet) presented no toxic manifestations (Combs, 1997).

- The Expert Group on Vitamins and Minerals determined that 910 μg/day was its LOAEL, and they applied an uncertainty factor of 2 to calculate its Safe Upper Level for selenium of 450 μg/day (Expert Group, 2003).

- Schrauzer independently concluded that extradietary selenium supplementation of 200 μg/day was generally considered to be safe and adequate for adults of average weight who subsisted on the typical American diet. He estimated that the average adult would consume a total of 280-350 μg Se/day, a figure which compared favorably with EPA's oral RfD. Schrauzer also noted that prolonged daily selenium intakes of 750-850 μg/day do not produce adverse effects, supporting his contention that selenium supplementation of 200 μg/day provides a wide margin of safety (Schrauzer, 2001).

- Whanger's research on the selenium-cancer relationship led him to endorse the Food and Nutrition Board upper safe limit for selenium of 400 μg/day. Nonetheless, he reported that human subjects consuming up to 600 μg Se/day appear to have experienced no adverse clinical symptoms (Whanger, 2004).

- On-going clinical testing of SelenoExcell® by the University of Arizona team, with Reid, Stratton, and Marshall continuing the studies previously spearheaded by Clark, have expanded the daily human doses for the selenized yeast up to 1600 and 3200 μg. After 12 months of exposure at these high levels, no serious toxicities were observed, and it was concluded that humans can well tolerate daily doses greater than 400 μg without serious toxicity.

- Most recently, EFSA's expert panel provided its opinion as to the safety of selenium-enriched yeast when derived from sodium selenite and incorporated into foods and food supplements for particular nutritional uses within the general population, and they concluded that consumption levels providing selenium levels of about 100 μg/day does not present a safety hazard. This Panel specifically acknowledged the UL of 300 μg/day as part of their evaluation, along with safety reviews by several other international bodies and governmental agencies (European Food Safety Authority, 2008).

From the substantial body of published scientific literature summarized above, it is apparent that total daily selenium exposures to 200 μg should be well tolerated. Since the typical American adult ingests about 100 μg of selenium as part of the present daily diet, incorporation of an additional 100 μg of selenium from added SelenoExcell® in the foods targeted by CSI would be safe. Furthermore, an additional 200 μg of selenium from the addition of SelenoExcell® to the designated foods to allow for the 90th percentile

consumer (FDA, 1995) still yields a dietary level that is well below the ADI. Such levels of dietary high-selenium yeast take into account Rayman's expressed uncertainties about optimal intakes for selenium (Rayman, 2008).

Such a level of selenium should be well tolerated without toxic manifestations, in light of the NOAELs and safe upper levels that have been calculated from human data with uncertainty factors of 2 or 3 applied to the NOAEL. This is further bolstered by the additional remarks of several experts who have tested larger selenium doses on humans, including some that specifically incorporate SelenoExcell® at levels more than 20-30 times the selenium level of 100 μg that is presently under consideration. In short, the additional usage of 100 μg/day of selenium from SelenoExcell® as an added food ingredient is considered to be a safe level when factoring in the conservative estimates applied by several knowledgeable experts, including the 2008 opinion provided by EFSA which also views the 100 μg/day of selenium from selenium-enriched yeast to present no safety concerns.

The question was raised as to the possibility that consumption of high-selenium yeast would trigger allergic reactions in sensitive consumers. This aspect was addressed specifically in Section III., and no evidence was uncovered from the scientific literature or from direct contacts with knowledgeable organizations who are professionally interested in allergic manifestations in humans. In addition, the EFSA Panel of 2008 specifically reviewed this topic, as well, and concluded that there was no particular concern about a subpopulation vulnerability that would not be adequately addressed through food product labeling practices.

In 2007, a scientific article was published that raised the concern that long-term consumption of dietary selenium could increase the risk of type 2 diabetes in some individuals. It was acknowledged by the authors that the findings were preliminary and that the study had design limitations. One of the co-authors, USDA's Dr. G. F. Combs, Jr., extended the investigation into the possible linkage between type 2 diabetes and consumption of supplemental selenium. In 2008, he reported the results of his year-long investigation that did not demonstrate a cause and effect correlation. Thus, the most recent focused effort by a highly qualified scientist has dispelled the allegations of type 2 diabetes triggered by selenium consumption.

The overall safety considerations should extend to the subpopulation that resides in geographic areas that have naturally higher soil levels of selenium. The question may arise as to whether or not this subpopulation might consume levels of Se that are above the commonly accepted ADI or UL. This possible concern was of more significance in years gone by when food distribution practices in the US were more regionalized than is the practice today. As noted in Section II.A, US consumers are increasingly exposed to foods that are produced and distributed nationally with a corresponding diminished reliance on locally or regionally produced foods. This practice of increased reliance on nationally produced foods serves to mitigate concern that subpopulations residing in areas with high selenium levels might ingest excess dietary selenium. In fact, some of the foods that would be fortified with SelenoExcell® would actually replace foods regionally produced from high-selenium crops. Such replacement could actually result in a reduction in dietary selenium for those living in high selenium regions.

Furthermore, in those cases where sustained, excessive selenium intake may lead to selenosis, such effects are mild, readily observable, and are reversible. Thus, in the unlikely circumstance that the high-selenium yeast would contribute to significant intake in individuals already consuming elevated amounts of selenium, self-correcting action could be taken without residual harm to the consumer.

Our Expert Panel considered one remaining issue associated with conferring GRAS status on the high-selenium yeast that is to be added to designated foods to provide 100 μg Se/day. We must consider the specific selenocompounds that constitute SelenoExcell® since we know that not all selenocompounds exhibit identical safety or toxicity profiles. We have established that organoselenium compounds such as selenomethionine are not only more bioavailable than inorganic sources of selenium but they also exhibit diminished toxicity when compared with inorganic selenium sources as established by human and animal testing. In order to draw a conclusion that a particular material is safe, one must know WHAT that particular material is. It is risky to extrapolate safety or toxicity conclusions about one selenocompound to another. This conclusion would also hold when comparing one high-selenium yeast to another high-selenium yeast, even though the extent of chemical differences among such selenium yeasts are lessened since it has been established that selenomethionine is the predominant selenium species in the high-selenium yeasts.

The safety or toxicity profiles of all selenium compounds that have been subjected to the testing described in this document have been considered thus far. Such data provide an overarching framework that the proposed food uses of SelenoExcell® are safe.

An active area of selenium research over the past several years has been speciation studies where scientists attempt to elucidate the specific chemical entities that make up mixtures such as are commonly found with selenized yeasts. Table 4 summarizes the chemical composition of SelenoExcell®, and we see that about 85% of the selenium content has been identified as to its exact chemical form. This extent of characterization also applies to other selenized yeasts. What this means is that about 10-20% of the selenium could be present in some chemical forms that have not yet been determined conclusively. How does this compositional uncertainty impact the safety considerations?

A narrowed focus has been directed toward the specific chemical composition of SelenoExcell® since there are unique circumstances associated with its human food and supplement uses that override the compositional issues noted above. We have concluded that supplementing the diet with SelenoExcell® to provide 100 μg of selenium per day is GRAS, even without knowing with certainty nearly 15% of the chemical composition. SelenoExcell® was utilized in the Clark cancer study which spanned over ten years, and it continues to be the test subject in follow on studies administered by Marshall and Reid (Clark, et al., 1996; Clark, et al., 1998; Stratton, et al., 2003a; & Stratton, et al., 2003b). The past clinical experience with SelenoExcell® without apparent toxicity or noted adverse effects overrides the compositional uncertainties when drawing this safety conclusion. In this regard, the designation that SelenoExcell® as GRAS at a daily intake level that provides 100 μg of selenium is justified.

By considering all accumulated information as presented in Sections II. and III. and discussed in Section IV., the Expert Panel has determined that the "reasonable certainty of no harm" standard has been well-established. Furthermore, the data and studies upon which this determination was made are overwhelmingly generally available for evaluation. The conclusion of the Expert Panel is conferring GRAS status in concert with the specified food categories and use levels is in full harmony with the scientific views of numerous other expert bodies and scientists both within the US and from abroad, thus establishing that the stated conclusion fulfills the consensus requirement that is needed.

V. CONCLUSIONS[17]

The cumulative scientific information on high-selenium yeast, selenomethionine, and SelenoExcell®, specifically considering the human experiences and associated testing, pertinent animal test results, anticipated human consumption levels, and germane supporting information, provide the basis for the conclusion that a daily SelenoExcell® exposure as a nutrient supplement that provides selenium levels up to 100 µg for adults, with proposed food usage as summarized in Table 6, is generally recognized as safe. SelenoExcell® must be produced in accordance with GMP procedures and must comply with appropriate food grade specifications.

We have independently and collectively evaluated the above-referenced information and offer this GRAS declaration based on scientific procedures in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

In conclusion, adult human exposure to SelenoExcell®, as an ingredient added to selected food categories at a combined level that provides up to 100 µg per day of selenium is generally recognized as safe (GRAS).

Robert S. McQuate, Ph.D.

September 2, 2008

Richard C. Kraska, Ph.D., DABT

September 2, 2008

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[17] The credentials for the Expert Panel members are summarized in their respective resumes which can be found in Appendix D. Both Dr. McQuate and Dr. Kraska have extensive technical backgrounds in the evaluation of food ingredient safety; each worked within FDA's GRAS Review Branch earlier in their careers and subsequently continued such evaluations in the private sector.

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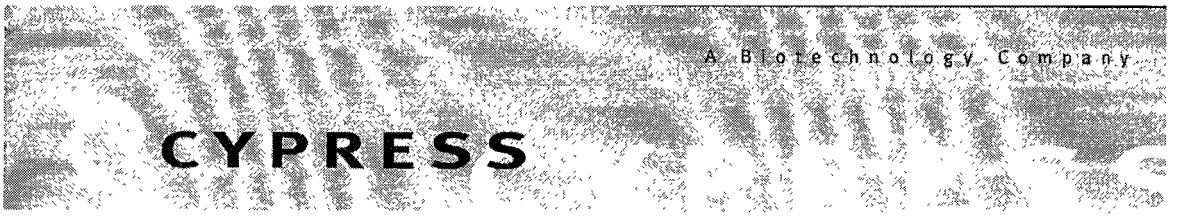
APPENDIX A

Cypress Systems Production & Quality Procedures for SelenoExcell®



SelenoExcell™ High Selenium Yeast Production Protocol

- **Cypress utilizes standard baker's yeast strains (Saccharomyces cerevisiae) for all production.**
  - ~ *These strains are recognized for food applications and are listed on the FDA GRAS list (Generally Regarded As Safe).*
- **Selected strain is derived from primary grown pure culture mother stock.**
  - ~ *Base stock derived from pure cultures routinely yield a higher quality and more consistent product than those utilizing secondary or waste stream yeast from the brewing of alcohol processes*
- **The specific strain selected by Cypress consistently yields a high protein product ranging from 50% - 55%.**
  - ~ *The majority of organic binding of the mineral composition occurs at various protein (amino acid) sites High protein strains are required to assure maximum binding sites for optimum uptake of desired mineral.*

    Note. Low protein (30 – 35%), secondary yeast from brewing process provides minimal protein receptors for organic binding
- **Fermentation is performed in precisely controlled aerobic fermentation vessels which closely maintain yeast growth, nutrient feed streams, dissolved oxygen, pH, temperature and the presence of alcohol which indicate lower than optimum growth performance.**
  - ~ *Close monitoring of key fermentation and growth parameters, yields a high quality, consistent finished product.*
- **Selected mineral composition (selenium) is introduced at various time intervals and growth parameters to assure proper uptake and maximum utilization for organic binding.**
  - ~ *This process assures that the mineral is only introduced during active growth (cell doubling) fermentation when maximum protein synthesis is occurring (i.e maximum utilization of mineral and increased organic binding).*

    Note: Duration of mineral yeast fermentation is 14 – 16 hours compared to a standard baker's yeast fermentation of 8 hours

5150 North 6th Street, Suite 156, Fresno CA 93710-7511 www.cypsystems.com Phone 559-229-7850 Fax 559-275-9007

- **Following active growth fermentation, mineralized yeast cream is washed and separated four times with a doubling (2x) of clean wash water.**
  - ~ *With maximum uptake of mineral during fermentation, this washing procedure assures that any remaining free mineral is properly washed away.*

    Note: Production commitment to this washing (4x with 2x clean water) requires extensive resources for equipment, processing time and wastewater treatment. This commitment is not common in other processes, but is the final assurance that SelenoExcell™ is free of inorganic forms of minerals

- **Yeast cream is held in cold storage prior to downstream processing.**
  - ~ *Cold storage provides proper holding condition to maintain yeast viability and protect organic binding of mineral.*

- **Prior to spray drying, yeast cream is pasteurized and brought to an inactive state.**
  - ~ *Enables product to achieve low micro counts required to meet or exceed established standards for human food grade products.*

- **Following pasteurization, yeast cream is spray dried.**
  - ~ *Spray dried mineralized yeast is uniformly blended into most dry powder formulations creating a homogenous mixture.*

- **SelenoExcell™ High Selenium Yeast is produced by a highly controlled method, which provides a 100% organically bound form of selenium with minimal batch to batch variations.**
  - ~ *As demonstrated by leading health and cancer research groups, formulators and consumers can rely on the consistent quality and performance of SelenoExcell™ to deliver the full composition of organically bound selenium, which most resembles that found in nature.*

- **Branded Trademark**
  - ~ *The product resulting from the original production protocol is currently trademarked as SelenoExcell. Specific labeling guidelines are available upon request.*

A Biotechnology Company

CYPRESS

Quality Assurance / Quality Control Program

By establishing a critical benchmark based on consistent high quality, we can assure customers and research groups that our product will enhance their product formulation. Therefore we have developed a comprehensive Quality Assurance / Quality Control (QA / QC) Program for the testing and reporting of Microbiology and Nutrient Analysis on all of our products.

The first stage in our QA / QC program is to use leading, independent laboratories for all testing on Cypress products. This practice lends confidence to Cypress, and our customers, that our products meet or exceed USDA requirements for food grade material.

Silliker Laboratories perform all microbiology testing on Cypress products. For over 25 years Silliker has been a leading, independent food testing organization. They have grown from a single lab in 1967, to an international network of laboratories that specialize in assessing the safety, quality, and nutritional value of foods. Silliker Labs are USDA and FDA certified in Microbiology.

All Cypress products are tested by Silliker Labs for the following: (included are USDA ranges for food grade materials and a typical Cypress product results).

Tested	USDA Food Range	Typical Cypress
Salmonella	Negative	Negative
E. Coli	Negative	Negative
Total Coliforms	< 10 / gram	< 1 / gram
Total Plate Count	< 7000 / gram	< 100 / gram
Yeast / Mold	< 50 / gram	< 10 / gram

In addition to the above tests, several times each year products are tested for Arsenic, Cadmium, Lead, and Mercury. Our results have been:

Arsenic	Non-detectable
Cadmium	< 1 mcg / gram
Lead	< 1 mcg / gram
Mercury	< 1 mcg / gram

Analysis to document nutrient content and organic binding is conducted by a FDA and USDA certified independent laboratory. Established testing methods have produced a 99% consistency in reported results.

Routine testing by an independent laboratory certifies that our products are absent of "free" and inorganic forms of supplemented minerals

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Test Methods

From sterile samples that are obtained during the drying process, a composite sample is created. All analyzed samples used for Nutrient and Microbial Analysis are taken from the composite sample.

Nutrient Analysis:

The following test methods are used to obtain required data on nutrient specifications prior to the sale of product.

Test	Method
Selenium Content	ICP Method
Organic Binding - Free Selenium	Methylene Blue Test
Moisture	Gas Column Chromatography

Microbial Analysis:

The following test methods are used to obtain required data on microbial specifications prior to the sale of product.

Test	Method
Coliform	MPN
E. Coli	U.S. Pharmaceutical Method
Yeast & Mold	PDA
Total Plate Count	SMA Total Plate Count Agar
Salmonella	EIA, enriched with lactose broth in the first step of the method

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APPENDIX B

Certificate of Analysis for SelenoExcell®

CYPRESS A Biotechnology Company

Manufacturer's Certificate of Analysis

SelenoExcell™ High Selenium Yeast

Lot Number: Se-71

Nutrient Analysis:	
Total Selenium:	1,255ppm
Protein:	50.4 %
Color:	Tan
P205:	2.92
Moisture:	4.1% %
Extraneous Material:	Negative
Microbiological Assay:	
Salmonella:	Negative
E. Coli:	Negative
Total Coliforms:	<0.3 /gram
Total Plate Count:	<10 /gram
Yeast/Mold:	<10 /gram
Staph. aureus:	Negative
Bacillus cereus:	Negative
Heavy Metals:	
Arsenic:	<1 MCG/gram
Cadmium:	<1 MCG/gram
Lead:	<1 MCG/gram
Mercury:	<1 MCG/gram
Particle Size:	
Bulk Density:	0.6515
Through 60 mesh	100%
Through 100 mesh	100%
Organically Bound Selenium:	Positive No Free Selenium

Manufacture Date: October 15th, 2004
Date of Expiration: October 15th, 2007

Country Of Origin Mexico

PESTICIDE FREE

These results are reported by:

Lon C. Baugh, Ph.D.
Quality Assurance
Fermentation Consulting, Inc.

Date: 8-11-2004

3381 North Bond Avenue, Suite 101, Fresno, CA 93726 www.cypsystems.com Phone 559-229-7850 Fax 559-225-9007

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APPENDIX C

Absence of Diabetes Indicators with Selenium Supplementation

USDA ARS United States Department Of Agriculture Agricultural Research Service

Research Project: Role of Dietary Selenium on Gene Expression, Cell Cycle and Molecular Mechanisms in Cancer Risk

Location: Grand Forks Human Nutrition Research Center

Title: Absence of Diabetes Indicators in a Selenium-Supplementation Trial

Authors

- Combs, Gerald
- Watts, Jennifer
- Johnson, Luann - UNIV OF NORTH DAKOTA
- Canfield, Wesley
- Davis, Cindy - NATL CANCER INSTITUTE
- Milner, John - NATL CANCER INSTITUTE

Submitted to: Journal of Federation of American Societies for Experimental Biology
Publication Type: Abstract
Publication Acceptance Date: January 31, 2008
Publication Date: April 15, 2008
Publisher's URL: http://www.fasebj.org
Reprint URL: http://www.fasebj.org
Citation: Combs, G.F., Watts, J.J., Johnson, L.K., Canfield, W.K., Davis, C.D., Milner, J.A. 2008. Absence of Diabetes Indicators in a Selenium-Supplementation Trial. Journal of Federation of American Societies for Experimental Biology. 22:696.4.

Technical Abstract: We conducted a yr-long intervention with selenium (Se) to characterize dose-response relationships of biomarkers of Se status. Volunteers from Grand Forks, ND, were screened by interview, medical history and clinical biochemistry; individuals with liver or renal dysfunction, uncontrolled hypertension, unmanaged diabetes, or BMI>40 were ineligible. Of those eligible, 261 (106 men, 155 women) were randomized to Se supplements (0, 50, 100 or 200 ug Se [as L-selenomethionine]/day). Baseline plasma Se levels were relatively high (141.5±23.7 ng/ml), with >50% in the upper quintile of the NHANES III cohort, and 6% in the range achieved only by Se supplementation in the NPC trial. As secondary analyses of those studies have suggested associations of plasma Se level and diabetes risk, we evaluated that relationship in our cohort. At baseline, 15 subjects reported having diabetes and/or using diabetic medications; they showed higher ($P<0.05$) fasting glucose levels (120±25 mg/dl) than subjects without apparent diabetes (88±9 mg/dl), but similar plasma Se levels (132.4±18.3 ng/ml vs. 142.6±23.7 ng/ml) with cases similarly distributed over the tertiles of baseline plasma Se. Subjects without apparent diabetes showed similar fasting glucose levels across those tertiles (low: 86.4±9.0 mg/dl; mid: 87.5±8.3 mg/dl; high: 90.2±10.1 mg/ml). These results do not support a relationship of Se status and diabetes risk.

Last Modified: 06/16/2008

United States Department of Agriculture
Research, Education, and Economics
Agricultural Research Service

June 18, 2008

Dr. Mark Whitacre
President, Cypress Systems, Inc.
3381 North Bond Ave., Suite 101
Fresno, CA 93726
Fax: (559) 225-9007

Dear Dr. Whitacre:

I am responding to your request of June 17 for my assessment of the paper by Stranges et al (Ann. Int. Med. 147:217-223, 2007) of which I was a co-author.

As you know, in that paper we reported observations from the Nutritional Prevention of Cancer (NPC) Trial that subjects treated with Se (Se-yeast providing 200 mcg Se/day) had an excess of type 2 diabetes. (This was the same cohort in which we found Se supplementation to reduce risks to cancer mortality and cancers of the prostate, colon-rectum and lung [Clark et al, JAMA 76:1957, 1995]). I'd point out that another research group (Bleys et al, Diabetes Care 30:829, 2007) made similar observations independently: they noted excess type 2 diabetes among subjects in the upper quintile of plasma selenium in the NHANES III cohort.

These the inferential value of these reports are limited by aspects of in their designs and/or methodologyies. These include that fact that each relied on subject-reported diagnoses and recorded medication use for ascertainment of diabetes status. Cases so identified were not confirmed (e.g., with measurements of fasting glucose, insulin sensitivity, etc.). In the NPC Trial, diabetes risk factors (e.g., BMI, waist circumference, etc.) were not considered in randomizing subjects to treatment, and no baseline measurements of risk factors were made to ascertain the relative risks of each treatment group. In light of these issues, and because we know that type 2 diabetes is frequently undiagnosed, I consider these results to be provocative, but certainly not established.

With this question in mind, we examined data from a trial that we recent completed at this Center. This was a double-blind trial in which men and women were randomized to 0, 50, 100 or 200 mcg Se as L-selenomethionine for a year. I would point out that more than half of our subjects showed baseline (pre-supplementation) plasma selenium levels corresponding to the upper quintile of plasma Se in the NHANES III cohort. We reported the pertinent results at the Experimental Biology meetings this spring; they have been published only in abstract form to day. Briefly our findings are as follows.

ars

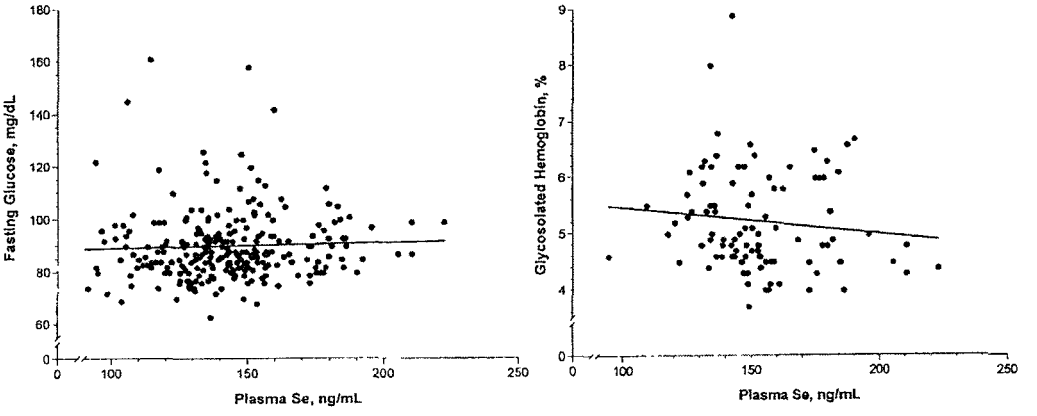
Northern Plains Area, Grand Forks Human Nutrition Research Center
Office of the Center Director
2420 2 Ave N, STOP 9034, Grand Forks, ND 58202-9034
Phone: 701-795-8456 FAX: 701-795-8230 Email. Gerald Combs@ars usda.gov

Of 259 randomized subjects, 15 reported having been diagnosed with diabetes and/or using diabetic medications (medical management of existing conditions was an eligibility criterion). As expected, diabetes patients showed significantly greater baseline fasting glucose levels than subjects without diabetes (Table 1). Nevertheless, cases and non-cases showed similar plasma Se levels, and cases were regularly distributed over the tertiles of baseline plasma Se.

Table 1

subjects (n)	Se, ng/ml	glucose, mg/dl
diabetic (15)	132±18	120±25
non-diabetic (244)	142±24	88±9*

Subjects without apparent diabetes showed similar fasting plasma glucose levels across those same tertiles. Fasting plasma glucose and % glycated hemoglobin (HbA1c) were not related to plasma Se level (see figures).



Thus, our recent results, produced with appropriate methods, do not indicate a relationship of Se status and diabetes risk. While we recognize the inferential limitations inherent in a small study such as this, it is also the case that these are the most robust data relevant to the question of whether selenium status may be related to diabetes risk. Thus, it is our plan to continue to address this hypothesis in further studies; but for the moment, I consider the supporting evidence very weak.

Sincerely,

Gerald F. Combs, Jr., Ph.D.
Center Director

APPENDIX D

Credentials of Expert Panel

ROBERT S. MCQUATE

Email:

WORK HISTORY

2006 – Present	Co-Founder & CEO, GRAS Associates, LLC, Bend, OR
1988 -- Present	President & CEO, R. S. McQuate & Associates, Inc., Bend, OR
2005 – 2006	Chemistry Professor, Truckee Meadows Community College, Reno, NV
2000 -- 2005	Senior Vice President, Scientific & Regulatory Affairs, AminoPath Labs, LLC, Portland, OR
1998 -- 2002	Board Member & Consultant, National Institute of Standards & Technology, Advanced Technology Program, Gaithersburg, MD
1986 – 1996	Executive Director, Advanced Science & Technology Institute, Eugene & Corvallis, OR
1991 -- 1992	Adjunct Professor, Food Science & Technology, Oregon State University, Corvallis, OR
1983 -- 1986	Science Director, National Soft Drink Association, Washington, DC
1980 -- 1983	Senior Regulatory Scientist and Group Leader of Regulatory & Nutrition, The Dial Company, Inc., Scottsdale, AZ
1977 -- 1980	Consumer Safety Officer, Food and Drug Administration, Center for Food Safety & Applied Nutrition, Division of Food and Color Additives, Washington, DC
1974 -- 1977	Assistant Professor of Chemistry, Willamette University, Salem, OR

EDUCATION

- Postdoctoral Research Fellow with Professor R. G. Wilkins, New Mexico State University, Las Cruces, NM
- Ph.D. in Chemistry, The Ohio State University, Columbus, OH
- B.S. in Chemistry with Honors, Lebanon Valley College, Annville, PA

PROFESSIONAL EXPERIENCE

CONSULTING SERVICES

CEO, GRAS Associates, LLC; President & CEO, R. S. McQuate & Associates, Inc.

- Generate regulatory strategies to achieve food ingredient marketplace acceptance for clients.
- Interpret FDA's Red Book on food additive & GRAS safety evaluations in designing food ingredient testing regimens.
- Provide food ingredient safety evaluations, focusing on independent GRAS evaluations, food & color additive petitions, new dietary ingredient compilations, and associated FDA submissions.
- Serve on Expert Panels with particular orientation toward chemical composition and food ingredient specifications.
- Utilize quantitative risk assessment tools to ascertain likely food ingredient risks.
- Assess compositional information on ingredients---including complex natural products---to determine safety influences by various constituents and contaminants.
- Extract present day and historical consumer exposure information on foods to support clients' projected ingredient usage.
- Extensive writing & editing of technical papers and reports---including authoring food additive petitions, GRAS

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Notifications and ingredient safety dossiers---to support client marketing initiatives.

- Provide aggressive interpretation of scientific documentation to support client labeling and advertising representations.
- Serve as liaison with FDA scientific/regulatory staff in pursuing clarification of technical regulatory topics of concern to clients.
- Utilize negotiation skills to achieve mutually acceptable problem resolution.
- Develop proactive regulatory positions to avoid adverse regulatory compliance conditions by drafting client-specific Product Recall Procedures and FDA Inspection Procedures.

UNIVERSITY EXPERIENCE

Executive Director, Advanced Science & Technology Institute

- Managed industry-university interface program on behalf of University of Oregon, Oregon State University, Oregon Health & Science University and Portland State University.
- Facilitated linkages between university research community and private sector, working with over 500 faculty members to yield consulting contracts, industrial research sponsorship, technology licensing and business start-ups.
- Aggressively marketed faculty expertise, universities' technologies, and research capabilities through network of contacts, Internet, publications, and conferences.
- Represented universities in broad-based statewide and regional economic development initiatives.
- Strategic planning and program implementation; managed staff of 5 to 8.

Faculty, Willamette University, Oregon State University, & Truckee Meadows Community College

- Taught introductory and upper level chemistry lecture and laboratory courses.
- Conducted independent research in molecular biology, enzymology, and metal ion catalysis.
- Successfully generated external grant funding to support research students and acquire equipment.
- Provided food safety guidance to industry, including drafting GRAS evaluations.
- Published scientific and chemical education papers.

PRIVATE SECTOR EXPERIENCE

Technical Management, The Dial Company & National Soft Drink Association

- Managed 5-person technical regulatory group with corporate responsibility for compliance with FDA, USDA, EPA, FTC, OSHA, CPSC, and NRC.
- Creatively interpret regulations to favorably impact company revenues by over $4.2 M annually.
- Special focus on product and ingredient safety; formulated regulatory strategies in anticipation of and in response to agency positions; applied quantitative risk analysis to product safety considerations.
- Provided regulatory support and training to Manufacturing and QA on Good Manufacturing Practices requirements.
- Teamed with Marketing by evaluating advertising, product claims, and labeling for compliance.
- Assessed university research proposals in response to industry solicitations for funding.
- Served as liaison for industry interests on food ingredient safety before FDA officials.
- Active participant on 10 technical committees dealing with food and food ingredient safety within the International Life Sciences Institute (ILSI).
- Served as industry spokesperson with media on technical topics such as NutraSweet addition to soft drinks.

GOVERNMENT EXPERIENCE

Staff, Food & Drug Administration

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- FDA representative with regulated food industry officials.
- Managed safety evaluations of food and color additives and GRAS ingredients among FDA scientific divisions

and with legal staff.

- Served on initial FDA technical team to establish and implement the "Cyclic Review" safety assessment of food additives.
- Generated food safety notices, proposals, and regulations.
- Evaluated complex net weight food labeling and compliance issues and formulated agency position for Commissioner.
- Participated on special FDA Food Labeling Task Force to develop total food label requirements.
- Formulated recommended agency policy on iron fortification practices in light of bioavailability nutritional concerns.

PROFESSIONAL AFFILIATIONS

American Chemical Society Institute of Food Technologists

BOARD AND COMMITTEE MEMBERSHIPS

- Member of Institute of Food Technologists' Expert Panel to Assess Food Chemical Safety Evaluation Practices (2007-present)
- Proposal Evaluation Boards in Chemistry & Materials within the Advanced Technology Program, National Institute of Standards & Technology (1998-2002)
- External Evaluator, Kansas Technology Enterprise Corporation, Higuchi Biosciences Center (2001)
- Judge, Ohio State University Business Plan Competition (2001)
- Board of Directors - Universal Pulping, Inc. (1996 - 2004)
- Scientific Advisory Committee - Bainbridge Technology Group, Ltd. (1991 - 2000)
- Board of Directors - Regional Council of Project SBIR West (1994 - 1996)
- Board of Directors - Oregon Environmental Technology Association (1994 - 1995)
- Co-Director – Oregon Governor's Task Force on Technology Transfer (1991 - 1992)
- Board of Directors - LEAP, Inc. (1988 - 1994)
- Board of Directors - Oregon Biosciences Association (1991 - 1993)
- Board of Directors - BioForum (1988 - 1991)
- Oregon Governor's Biotechnology Industry Advisory Council (1988)

HONORS AWARDS AND FELLOWSHIPS

- Governor Barbara Roberts Certificate of Appreciation - Task Force on Technology Transfer (1993)
- Governor Neil Goldschmidt Letter of Commendation - Biotechnology Industry Advisory Council (1988)
- FDA Award of Merit from FDA Commission Jere Goyan (1980)
- Letter of Commendation from FDA Commissioner Donald Kennedy (1979)
- Seven Research Grants Awarded as Faculty Member at Willamette University (1974 - 1977)
- National Science Foundation - Graduate Research Fellowship, The Ohio State University (1971 - 1973)
- Graduated with Honors, Lebanon Valley College (1969)
- Petroleum Research Fund - Undergraduate Research Fellowship, Lebanon Valley College (1967 and 1968)
- Dean's List Student, Lebanon Valley College (1966 - 1969)
- Salutatorian, South Lebanon High School, Lebanon, PA (1965)

Richard C. Kraska
Chief Operating Officer and Co Founder

Curriculum Vitae

EDUCATION B.S., Chemistry; Providence College
Ph.D., Pharmacology; University of Minnesota

PROFESSIONAL CERTIFICATION Diplomate, American Board of Toxicology

EXPERIENCE 29 year in toxicology and regulatory affairs for industry and government in broad aspects of the chemical industry including food additives, foods, food contact materials, cosmetics, lubricants and fuels, coatings, defoamers, anti-microbial pesticides and pharmaceuticals.

GRAS ASSOCIATES, LLC
Bonita Springs, FL (2006 to Present)

Chief Operating Officer and Co Founder

- Serve as Lead Scientist and Panel Chair for GRAS determinations.
- Coordinate drafting and report review by chemists, toxicologists and scientists of other disciplines as needed.
- Ingredients reviewed include natural antioxidants, novel sources of dietary fiber, fats and oils and extracts from exotic fruit.

KRASKA CONSULTANTS, INC.
Bonita Springs, FL (2004 to Present)

Vice President and Principal

- Toxicology and Regulatory Consultant for a variety of lubricant, chemical, food processing companies and trade associations
- Offer services in Toxicology and Product Safety including FDCA, TSCA and FIFRA regulations and filings, International Hazard Communication Support, Product Stewardship, Expert Witness and Litigation Support
- Founder and Technical Consultant for the Defoamer Industry Trade Association
- Toxicology Consultant for the Independent Lubricant Manufacturers Association

THE LUBRIZOL CORPORATION
Wickliffe, OH (1987 to 2004)

MANAGER OF SPECIAL TOXICOLOGY AND REGULATORY PROJECTS (2001 to 2004)

- Toxicology and regulatory consultant for organic growth initiatives and new acquisitions.
- Coordinating [redacted] inhalation toxicology program on engines emissions with a novel diesel fuel formulation for registration with EPA under the Clean Air Act.
- Coordinating world wide implementation of compliance with revised European hazard communication regulations
- Consultant to Lubrizol defoamer, coating, process chemical, metalworking and lubricant businesses on regulations and toxicology
- Team member studying and planning implementation of sustainable development at Lubrizol.

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MANAGER OF TOXICOLOGY AND RISK ASSESSMENT (1987 – 2001)

- Provided leadership and management for corporate toxicologists and product safety specialists.
- Direct responsibility for toxicology testing and evaluation of all Company specialty chemicals and products.
- Manage [redacted] annual toxicology and environmental testing budget for regulatory approvals and product stewardship.
- Lead consultant for business units on novel regulatory approvals, product stewardship and risk evaluation.
- Developed and institutionalized product risk assessment process for all Lubrizol businesses.
- Provide leadership role representing Company on trade association task groups involved in legislative and regulatory advocacy.
- Co-team leader for development and implementation of award -winning expert system for writing MSDSs from a product safety database.

BP AMERICA INC (formerly THE STANDARD OIL CO)
Cleveland, OH (1985-1987)

MANAGER OF PRODUCT SAFETY AND REGULATORY COMPLIANCE

- Assumed responsibility for assuring all Company products complied with federal regulations (TSCA, FIFRA, FDCA, USDA).
- Coordinated and expedited all regulatory submissions for premarket approval, reporting rules and rulemaking comment.
- Conscientiously developed Company Product Safety Policies and Manual.
- Critically evaluated Corporate Hazard Communication Program in a decentralizing company.
- Successfully initiated labeling program to comply with OSHA Hazard Communication Standard.

AMERICAN CYANAMID COMPANY, CHEMICALS GROUP
Wayne, NJ (1983-1985)

MANAGER OF TOXICOLOGY PROGRAMS

- Wide range of responsibility for recommending, contracting, monitoring and evaluating mammalian, genetic and aquatic toxicology studies for chemical products.
- Responsible for (b)(6) total contract value for testing, quality assurance and consultants.
- Effectively guided regulatory staff in strategy and data requirements for premarket approvals.
- Successfully orchestrated targeted research programs for mechanistic studies on key chemicals for aquatic and mammalian toxicity.
- Actively represented Company in a wide spectrum of trade association activities.

FOOD AND DRUG ADMINISTRATION
Washington, DC (1977-1983)

GRAS Review Branch
Division of Food and Color Additives

SUPERVISORY CONSUMER SAFETY OFFICER
(1981-1983)

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- Successfully managed group of 3-4 professionals in regulatory program to implement expert panel reviews of GRAS list food ingredients.
- Projects of responsibility included salt, caffeine, BHA, BHT, cellulose, enzymes, rapeseed oil, vitamins, iron, manganese and zinc salts.
- Co-directed agency expertise on toxicology, chemistry, law and policy to propose regulatory action on food uses of DSS. Negotiated consistency with Bureau of Drugs proposal on OTC and Rx uses.

- Advised Branch Chief in matters of policy, consistency and personnel.
- Interacted with industry regarding regulatory opinions and new product approvals.

Petitions Control Branch
Division of Food and Color Additives

CONSUMER SAFETY OFFICER (1977-1981)

- Coordinated scientific review and regulatory response to review food additive petitions submitted by industry for direct additives and food packaging materials.
- Scientific and historical expert for General Counsel, U. S. Attorney and Department of Justice for legal proceedings on cyclamate.
- Expert on food/drug interface of vitamins and dietary supplements.
- Analyzed quality of critical studies on aspartame and served on GLP review committee
- Served as Bureau representative in Interagency Regulatory Liaison Group on phthalate plasticizers.
- Assistant to Bureau Director on advocacy activities on behalf of U.S. industry for WHO programs

PUBLICATIONS

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water-methanol emulsion emissions Toxicology & Industrial Health Vol 22 In Press

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, Naas DJ, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water emulsion emissions. Inhal Toxicol 17: 851-70 (2005)

Kraska, RC , Industrial Chemicals. Regulation of new and existing chemicals. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Kraska, RC . and Hooper DH, Industrial Chemicals. Hazard Communication, exposure limits, labeling and other workplace and transportation requirements under OSHA, DOT, and similar authorities around the world. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Strother, DE, Mast RW, Kraska RC, Frankos V Acrylonitrile as a carcinogen. Research needs for better risk assessment. Ann NY Acad Sci 534:169-78 (1988)

Petersen DW, Kleinow KM, Kraska RC, Lech JJ Uptake, disposition and elimination of acrylamide in rainbow trout Toxicol Appl Pharmacol 80: 58-65 (1985)

Mast RW, Jeffcoat AR, Sadler BM, Kraska RC and Friedman MA Metabolism, disposition and excretion of [C14] melamine in male Fischer 344 rats. Food Chem Toxicol 21: 807-810 (1983)

SPEAKER Talks given on following topics at national meetings, seminars and workshops

GRAS Criteria
REACH and GHS Regulations
HPV Toxicology Testing
Risk Assessment and Risk Management
Lubricant Additive Safety
Trade Association Environmental Activism
Product Deselection Lists
MSDS Expert Systems
Confidential Business Information under TSCA
TSCA Section 12(b) Compliance

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TRAINING COURSES Training courses given to business, research and legal groups at Lubrizol
- General Regulatory Overview
- TSCA New Chemicals
- FDA Food Additive Requirements
- Product Regulatory Law Course (TSCA, FDCA, OSHA)

Trainer, Toxicology Module, Metalworking Fluids Certificate Course (2005-2006)

TRADE ASSOCIATION ACTIVITIES

Chemical Reporting Task Group (1983-1998)
Chemical Manufacturers Association
Chairperson (1997-1998)

Safety, Health, Environmental and Regulatory Affairs Committee, Independent Lubricant Manufacturers Association (1997 to present)
- Vice chairperson (2001-2002)
- Chairperson (2003-2004)
- Toxicology consultant (2006)

Oversight Committee, Metalworking Fluid Product Stewardship Group, Independent Lubricant Manufacturers Association (1997-2004)

Health Environmental and Regulatory Task Group, Petroleum Additives Panel (1997-2002)
- Chairperson, Sensitization Work Group (1999 to 2002)

Biocides Panel, AEATF II Protocol Committee and Technical Committee (2003-2006) Team Leader for Metalworking Study (2005-2006)

Defoamer Industry Trade Association, Founder and Technical Consultant (2005-2006)

PROFESSIONAL SOCIETY MEMBERSHIPS

Society of Toxicology (SOT)
American Standards and Testing Methods (ASTM)
Society of Tribology and Lubrication Engineers (STLE)
Regulatory Affairs Professionals Society (RAPS)
Roundtable of Toxicology Consultants (RTC)

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SUBMISSION END

GRAS ASSOCIATES, LLC

20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

AM

September 8, 2008

Dr. Robert L. Martin
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for High-Selenium Yeast

Dear Dr. Martin:

I realize that I neglected to sign and date Section I.A in issuing the revised GRAS notification for High-Selenium Yeast tha was submitted on September 3.

Enclosed please find three copies of page 4 that have been signed and dated.

I apologize for the oversight.

Sincerely,

(b)(6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosure: Signature Page: GRAS Notification – High Selenium Yeast (in triplicate)

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

High-selenium yeast (*Saccharomyces cerivisiae)*, meeting the specifications described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by experts qualified by scientific training and experience; it is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the ingredient's intended use in foods.

Signed:

(b)(6)

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

Date: 9-03-2008

B. Name and Address of Notifier

Cypress Systems, Inc.[2]
3381 North Bond Avenue, Suite 101
Fresno, CA 93726

As the notifier, Cypress Systems, Inc. accepts responsibility for the GRAS determination that has been made for high-selenium yeast as described in the subject notification; consequently, high-selenium yeast meeting the conditions described herein is exempt from pre-market approval requirements for food ingredients.

C. Common Name and Identity of the Notified Substance

High-selenium yeast; also see Sections II.B and II.C.

[1] See 62 FR 18938 (17 April 1997).
[2] Cypress Systems, Inc. ("CSI") produces and sells SelenoExcell®, a high-selenium yeast product.

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20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

FAX TRANSMISSION

TO: Dr. Carrie McMahon

FROM: Robert McQuate

SUBJECT: High-Selenium Yeast GRAS Notification

DATE: September 19, 2008

Fax #: 301-436-1202

Total # Pages: 2

nments:

Attached is the GRAS Exemption Claim page that contains the date and my signature pertaining to the above-referenced GRAS notification.

The original signed pages were sent via US mail to Dr. Martin's attention.

Thank you.

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

High-selenium yeast (*Saccharomyces cerivisiae)*, meeting the specifications described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by experts qualified by scientific training and experience; it is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the ingredient's intended use in foods.

Signed:

(b)(6)

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

Date: 09-03-2008

B. Name and Address of Notifier

Cypress Systems, Inc.[2]
3381 North Bond Avenue, Suite 101
Fresno, CA 93726

As the notifier, Cypress Systems, Inc. accepts responsibility for the GRAS determination that has been made for high-selenium yeast as described in the subject notification; consequently, high-selenium yeast meeting the conditions described herein is exempt from pre-market approval requirements for food ingredients.

C. Common Name and Identity of the Notified Substance

High-selenium yeast; also see Sections II.B and II.C.

[1] See 62 FR 18938 (17 April 1997).
[2] Cypress Systems, Inc. ("CSI") produces and sells SelenoExcell®, a high-selenium yeast product.

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AM

Mcmahon, Carrie

From: Bob McQuate [McQuate@Gras-Associates.com]
Sent: Tuesday, December 30, 2008 3:21 PM
To: Mcmahon, Carrie
Subject: NCI SELECT Clinical Trial
Attachments: Dec Letter SELECT.doc

Dear Dr. McMahon,

Attached is a letter that summarizes information that we discussed recently on the actions taken by NCI on the Selenium and Vitamin E Cancer Prevention Trial.

In particular, our assessment of the safety considerations is such that GRAS status is warranted for the subject high-selenium yeast with the specified food uses and at the designated food use levels.

Please let me know if you have any remaining questions.

Sincerely,

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Attachment:
Dec letter SELE

GRAS ASSOCIATES, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

December 30, 2008

Dr. Carrie McMahon
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notice No. GRN 000260

Dear Dr. McMahon:

Since submitting the high-selenium yeast GRAS notification to FDA on behalf of Cypress Systems, Inc. which was filed on September 12, 2008 as GRN 260, we have learned of additional developments regarding clinical testing results associated with selenomethionine. We wish to bring this information to your attention and share our perspective on the safety ɔlications.

The National Cancer Institute (NCI) has funded the Selenium and Vitamin E Cancer Prevention Trial (SELECT), which is a multi-year, multi-center clinical investigation into the potential role of prostate cancer prevention by utilizing certain forms of selenium and vitamin E supplementation, taken alone or together. Recently reported results revealed that the cancer prevention study exhibited no benefits from the supplementation (see www.cancer/clinicaltrials/digestpage/SELECT).
(.gov)

We recognize that health benefits or the lack thereof are not factored into GRAS or food additive safety evaluations, and we will not address this aspect further regarding GRN 260.

Additional testing results potentially impacting safety were reported; it was suggested that individuals in the selenium test group may experience increased risk of type 2 diabetes mellitus (at a level of 10%) versus those individuals in the placebo group who were reported to experience a 9.3% increased risk of developing type 2 diabetes. In reporting this result, NCI stressed that the slight increased incidence of type 2 diabetes in men was **not statisically significant and might be due to chance**.

In reviewing NCI's comments and the recently-announced pre-publication addressing the SELECT actions which is to appear in JAMA in January 2009 (see Lippmann, S.M. et al., "Effect of Selenium and Vitamin E on Risk of Prostate Cancer and Other Cancers: TheSelenium and Vitamin E Cancer Prevention Trial (SELECT)," JAMA, published online December 9, 2008 (doi:10:1001/jama.2008.864)), clear reference was made to the non-statistically significant increased risk of type 2 diabetes associated with the selenium group.

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The question of increased risk of type 2 diabetes due to increased selenium intake was discussed in Sections III. and IV. of ‹N 260. Of particular importance was the finding from USDA's Dr. Gerald Combs, a prominent scientist who has been investigating physiological effects of selenium for most of his professional career, that increased selenium intake was not associated with increased type 2 diabetes. The results of a year-long investigation directed by Dr. Combs have been

published, and the authors report that **these results do not support a relationship between selenium status and diabetes risk**.

In light of the direct investigation by Dr. Combs as previously noted and the NCI report that acknowledges that the type 2 diabetes findings are not statistically significant and could be due to chance, we wish to reinforce our conclusion that high-selenium yeast (SelenoExcell®) consumed at a daily consumption level of 100 µg for the food uses designated in Table 6. as found in Section II.G.1 is GRAS.

If you wish to discuss this matter further, please feel free to contact me.

Sincerely,

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Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC.

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AM

Mcmahon, Carrie

From: Bob McQuate [McQuate@Gras-Associates.com]
Sent: Friday, January 16, 2009 1:40 PM
To: Mcmahon, Carrie
Subject: RE: GRN 260: high-Se yeast

Carrie,

I contacted Paul Willis, CEO of Cypress Systems, regarding your question on selenium content of commercial baker's yeast. He spoke with Dr. Lon Baugh, Cypress Systems' Director of Fermentation and Quality Control, who reported that the selenium level in commercial baker's yeast is less than 5 μg/g (0-5 μg/g). Typically, the selenium content is found to be "trace" or "not detected," but the commercial baker's yeast is produced using beet or cane sugar which is the probable source of the trace amounts sometimes found. Dr. Baugh has over 35 years of experience in fermentation/yeast production, and he was the individual who developed the high-selenium yeast, SelenoExcell, that is produced and sold by Cypress Systems.

I hope this adequately responds to your question.

Best regards,

Bob
Robert S. McQuate, Ph.D.
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541-678-5522
mcquate@gras-associates.com
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From: Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
Sent: Friday, January 16, 2009 8:14 AM
To: Bob McQuate
Subject: GRN 260: high-Se yeast

Bob -

For your information, our evaluation of GRN 260 (high-selenium yeast from Cypress Systems, Inc.) is progressing smoothly. However, I do have one quick question: CSI provides specifications for food-grade high-selenium yeast with target levels for selenium (1,140 – 1,260 μg/g) but does not provide information about how this compares to the typical range of selenium in traditional baker's yeast. What is the range typically found in commercial baker's yeast?

Regards,

Carrie

Carrie H. McMahon, Ph.D.
Consumer Safety Officer

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U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

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